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An investigation of the effects of thermal and non-thermal processing methods on Polyacetylenes from Apiaceae

A thesis submitted for the degree of Doctor of Philosophy by


School of Natural Sciences – Discipline of Biochemistry

National University of Ireland, Galway

Head of Discipline: Dr. Michael Carty
Head of School: Dr. Heinz-Peter Nasheuer
Supervisors: Dr. Maria Tuohy, Dr. Nigel Brunton

September 2011
Declaration of Originality

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Ashish Rawson

Signed:

Date: _______________________________
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Summary

Polyacetylenes are a group of phytochemicals that have attracted significant interest in recent years due to their range of potential health-promoting bioactivities however, little is known about the effects of food processing on them. In addition, their low abundance and relative instability requires rapid and very sensitive methods for analysis and characterization. Therefore the present work demonstrated the potential of ESI-MS, in multiple reaction monitoring (MRM)-quantitative methods for characterization of polyacetylenes and polyacetylene degradation.

Polyacetylenes in water immersion cooked carrot disks were significantly reduced at 50-60°C, but levels were higher at 70-100°C (p < 0.05) than in raw unprocessed samples. Blanching (95 +/- 3°C) prior to sous-vide (SV) processing (90°C for 10 min) of parsnip disks showed that blanching had the greatest influence on the retention of polyacetylenes. Subsequent chill storage (anaerobic conditions) resulted in a significant decrease in FaOH levels (p < 0.05) although no change in FaDOH levels was observed (p > 0.05). FaDOH was particularly susceptible to degradation during aerobic storage following blanching. Oxidized forms of FaOH, e.g. falcarindione, dehydrofalcarinol, dehydrofalcarinone were detected in thermally processed samples. Boiling and roasting decreased levels of all polyacetylenes in fennel bulb. And the presence of hydroxymethylfurfural was confirmed in roasted samples of fennel. Kinetic analysis of high pressure-temperature (HPT) processing of carrot disks revealed that FaDOAc was the most barosensitive while FaDOH was the most thermosensitive. Nonetheless, HPT yielded higher retention of polyacetylenes than SV-processing. Ultrasound pretreatment followed by hot air drying (UPHD) was shown to be a promising technique for retention of polyacetylenes and carotenoids in carrot disks in comparison to blanching followed by hot air drying.

In fresh cut products unit operations (mainly peeling) decreased polyacetylene retention, due to the high polyacetylene content of peels. Washing, after minimal processing, reduced polyacetylene content but retention was relatively high during storage and higher in parsnips than in carrots. Freezing and frozen storage of carrot disks revealed that blast frozen carrot disks retain higher amounts of polyacetylenes compared to their slow frozen counterparts. The texture and colour were also found to decrease during frozen storage.
Acknowledgements

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1. Introduction and literature review

1.1. General overview and context for the research

The EU is a major producer of fresh vegetables. Total EU production of fresh vegetables was 62 million tonnes in 2009, 3% up on the previous year (AMI, 2010). The most important vegetables in terms of production were tomatoes, onions and carrots. Italy and Spain had the largest vegetable production among EU Member States. Italy produced around 11.6 million tonnes of vegetables, while Spain produced approximately 9.9 million tonnes. The bulk of fresh vegetable production was concentrated in a few Member States. For example, two thirds of all the tomatoes produced in the EU originated from Italy and Spain in 2009, whilst 43.6% of the onions produced in the EU came from the Netherlands and Spain. (EuroStat, 2010).

Ireland is a high-income country with a well-developed food market. Potato, carrot, turnip, cabbage etc are the most popular vegetable in Ireland. In the year 2009, Ireland produced around 37 thousand tonnes of potatoes, 2.5 thousand tonnes of carrot and turnip and 4.5 thousand tonnes of cabbage and other brassica vegetables and ranked 69th in the world for the total production of carrot and turnip (FAO, 2011).

Vegetables are known to contain components (vitamins, essential minerals, antioxidants and prebiotics) with several types of health promoting actions and most of these have been evaluated in intervention studies. In general, health benefits from supplementation have been proved only for groups that had particularly low intake of these compounds, e.g. due to malnutrition, while supplemenations at high levels generally provided only minor additional improvements, and in some cases even showed adverse effects, as is the case for β-carotene (Omenn et al., 1996). However, the data from epidemiological studies shows sufficient linearity across the range of diseases (types of cancer and cardiovascular) to allow calculations of the benefits of substantial increases in the intake of fruits and vegetables to 400 g per day (van’t Veer et al., 2000) or even higher (Gundgaard et al., 2003). Historically carrots were known as an excellent source of vitamin A and important phytochemicals such as carotenoids and flavonoids. These phytochemicals are believed to help protect against various chronic diseases such as cardiovascular disease and cancer. However recently, researchers have identified another group of compounds in carrots that may
have health promoting properties, known as polyacetylenes. In particular falcarinol type polyacetylenes have been under continuous study due to their range of bioactivities which could provide health benefits to consumers.

Today’s busy lifestyle means that a high proportion of our vegetable intakes including carrots are consumed in processed forms. Many items are now retailed from the chill cabinet in gas flushed packs or as minimally/fully processed convenience vegetables/products with an extended shelf life. Many investigations into the effect of processing (both minimal and full) of the content of well known phytochemicals such as polyphenols and carotenoids have been carried out. However relatively little is known about the effect new and well established processing methods on polyacetylenes as their biological relevance has only emerged recently. To date, the application of heat is the most common method for processing food, because of its ability to kill microorganisms and inactivate spoilage enzymes (PPO, PME, etc). The use of thermal processing (water immersion, sousvide, hot air drying) has been favoured by the important technological developments experienced over the last few years together with the easier management of the equipment used (Soria and Villamiel, 2010). However, heat processing particularly under severe conditions may give rise to chemical and physical changes that impair the organoleptic properties and reduce the content or bioavailability of some bioactives (Patras et al, 2009a; Patras et al., 2009b). Therefore, there is a demand for mild processing technologies such as high pressure processing, power ultrasound etc. Recent interest in these technologies is not only to obtain high-quality food with “fresh-like” characteristics, but also to provide food with improved functionalities. As a result in the past decade novel emerging technologies such as high hydrostatic pressure treatment, power ultrasound have become established. High pressure processed products are now available world wide (Hendrickx, and Knorr, 2002). In addition to their possible beneficial effects on nutritional and bio-active content many of these novel technologies are more cost-efficient and environment friendly methods for obtaining premium quality foods and thus have led to their revival and commercialization (Piyasena et al., 2003; Vikram et al., 2005; Butz, and Tauscher, 2002). The overall aim of this study was to assess the effects of common manufacturing techniques on retention of polyacetylene in Apiaceae vegetable (carrot, parsnip, and fennel) products on the Irish Market with a view to recommending practices and protocols to maximise their retention. This work
examines the affect of minimal and full scale processing on levels of polyacetylenes in Irish Apiaceae vegetables (carrots, parsnips, and fennel).

1.2. Background
Epidemiological studies have shown that a high consumption of vegetables and fruits protect against certain types of cancer and other important diseases (Block et al., 1992; Steinmetz et al., 1996; Greenwald et al., 2001; Kris-Etherton et al., 2002; Maynard et al., 2003). Historically the health promoting effects of fruit and vegetables have been attributed to vitamins, minerals, fibres and antioxidants; however, there is a lack of strong epidemiological based evidence as to the exact components responsible for specific health benefits. Plants contain a myriad of bioactive components which can have a direct or indirect effect on living tissue in vitro and/or in vivo, which provide benefits for health, even though they are not essential nutrients (Brandt et al., 2004). Many of these components are in fact secondary metabolites, with known effects on human physiology and disease that have been identified through studies of plants used in traditional medicine. In addition, some bioactive compounds found in food plants are normally considered undesirable in human food due to their potentially toxic effects in high concentrations. However, a low daily intake of these “toxins” may be an important factor in the search for an explanation of the beneficial effects of fruit and vegetables on human health (Brandt et al., 2004). Polyacetylenes are examples of bioactive secondary metabolites that have been considered undesirable in plant foods due to their toxic and flavour properties. Some polyacetylenes are known to be potent skin sensitizers, and to be neurotoxic in high concentrations (Wittstock et al., 1995; Murdoch and Dempster, 2000), but have also been shown to have a pronounced selective cytotoxic activity against cancer cells (Hansen et al., 2003, Kobaek-Larsen et al., 2005). Some of these compounds are found in plants of the Apiaceae family, which in addition to some well-known medicinal and toxic plants also includes common food plants such as carrot, parsnip, fennel, and parsley.

1.2.1. Discovery, occurrence in all species
Polyacetylenes are widely distributed in nature, occurring in plants, fungi, lichens, moss, marine algae and invertebrates (Bohlmann et al., 1973; Christensen et al., 2008; Minto and Blacklock, 2008). So far, more than 2000 different polyacetylenes
are known of which the majority have been isolated from higher plants and in particular from the botanically related plant families Apiaceae, Araliaceae, and Asteraceae (Bohlmann et al., 1973; Christensen et al., 2008; Minto and Blacklock, 2008). The types of polyacetylenes found in plants include thiophenes, dithiacyclohexadienes, thioethers, sulfoxides, sulphones, alkamides, chlorohydrins, lactones, spiroacetal enols, ethers, furans, pyrans, tetrahydropyrans, isocoumarins, as well as aromatic and aliphatic acetylenes. The triple bond functionality and existence in R and S enantiomers of polyacetylenes makes these natural products to a very interesting group of compounds chemically and biochemically; their individual reactivity towards proteins and other bio-molecules may explain their wide variety of bioactivities.

1.2.2. Occurrence and biosynthesis in edible parts of plants

Polyacetylenes occur in a wide range of plants which are used for consumption. Table 1.1 summarizes the occurrence of polyacetylenes in the edible parts of plants. Of these carrots are the most commonly consumed root crop followed by parsnip and fennel. Research has indicated that of these three vegetables parsnips contains the highest amount of falcarinol and falcarindiol, followed by carrot which also contained falcarindiol-3-acetate, and fennel had the least amount of falcarinol and falcarindiol (Zidorn et al., 2005).

As reported by Bohlmann et al. (1973) and Hansen and Boll (1986), the biosynthesis of polyacetylenes of the falcarinol-type follows a well characterised pathway for aliphatic C<sub>17</sub>-acetylenes, with dehydrogenation of oleic acid leading to the C<sub>18</sub>-acetylenes crepenynic acid and dehydrocrepenynic acid, which are then transformed to C<sub>17</sub>-acetylenes by β-oxidation. Further oxidation and dehydrogenation leads to acetylenes of the falcarinol-type as outlined in figure 1.1.

1.2.3. Anatomical location of polyacetylenes in carrots

Czepa et al. (2004) studied the anatomical location of polyacetylenes in carrots, and demonstrated that highest concentrations of falcarindiol were present in the phloem and in the upper part of the root (33 mg/kg of fresh wt) and a lower amount (<20 mg/kg of fresh wt) in xylem tissue and the lower part. Distribution of falcarinol was found to be more homogeneous in the whole root, with average amounts of 25 mg/kg of fresh wt. Falcarindiol-3-acetate was found to be more concentrated in the upper
part of carrot root (15 mg/kg of fresh wt) than in the lower part (8 mg/kg of fresh wt), and no significant difference was seen between phloem and xylem tissue (Czepa et al., 2004). However in a similar approach Baranska et al. (2005) studied in detail the anatomical location of polyacetylenes in carrot disk by means of Raman spectroscopy. Accumulation of polyacetylenes was found in the outer section of the root, i.e. in the pericyclic parenchyma tissue close to the periderm, and to a higher extent in secondary phloem tissue close to the vascular cambium. However it was found that xylem parenchyma contained only a small amount of polyacetylenes. The localization of the polyacetylenes was attributed to the presence of vascular bundles in a young secondary phloem and to the pericyclic oil channels in the vicinity of the periderm. And these channels may be responsible for the transport and accumulation of polyacetylenes in the carrot root (Garrod et al., 1979; Baranska et al., 2005).

1.2.4. Function in carrots, parsnips and fennel
Plants contain a large number of different secondary metabolites, many of which display biological activity as natural pesticides with roles in plant defence against, for example, insects, fungi and other microorganisms. Falcarinol and falcarindiol are such secondary metabolites which have been identified as antifungal compounds in many Apiaceae plant species by inhibiting spore germination in different fungi at concentrations ranging from 20 to 200 µg/ml (Garrod et al., 1978; Kemp, 1978; Harding and Heale, 1980, 1981; Hansen and Boll, 1986; Olsson and Svensson, 1996; Christensen, 1998). Falcarinol was found to inhibit germination of Botrytis cinerea spores and its concentration was greatly increased when carrots were infected with this fungus (Harding and Heale, 1981). Falcarindiol was found to exhibit high toxicity towards Mycocentrospora acerina (Garrod et al., 1978) and carrot cultivars with high levels of this compound were less susceptible to this fungus (Olsson and Svensson, 1996). Falcarindiol-3-acetate is less active than either falcarinol or falcarindiol. In fact very little has been has been reported regarding its function however as it is always found present with other falcarinol type polyacetylenes in plants, and may exhibit some synergistic anti-fungal effects in plant. Polyacetylenes of the falcarinol-type tend to be present constitutively, indicating that they act primarily as pre-infection compounds in the species producing them, although some increase can be observed in response to infections (Garrod et al., 1978; Harding and Heale, 1980, 1981; Olsson and Svensson, 1996).
1.2.5. Toxicity

Experimental and chemical investigations have revealed that several plants containing falcarinol-type polyacetylenes are a potent contact allergens and are responsible for many allergic skin reactions (Hansen and Boll, 1986; Hausen et al., 1987; Oka et al., 1999; Hausen et al., 2001; Paulsen et al., 2010). However, this effect is not noticeable with vegetables belonging to the Apiaceae family, which may be due to the fact that these falcarinol-type polyacetylenes are present at lower concentrations in these food plants (Murdoch et al., 2000; Machado et al., 2002; Christensen and Brandt, 2006). The allergenic properties of falcarinol indicate that it is very reactive towards mercapto and amino groups in proteins, forming hapten-protein complexes (antigens). The reactivity of falcarinol towards proteins is probably due to its hydrophobicity and its ability to form an extremely stable carbocation (resonance stabilized) with the loss of water, thereby acting as a very strong alkylating agent towards various biomolecules (Kobæk-Larsen et al., 2005; Christensen et al., 2009; Purup et al., 2009).

1.2.6. Sensory aspects

Polyacetylenes of the falcarinol-type have been shown to impart sensory qualities in the edible parts of some plants e.g., carrot roots, parsnip roots etc. One such quality is bitterness, which is considered as an undesirable taste in carrots. However, Czepa et al. (2003) reported that bitterness in carrots is not due to presence of a sole compound but to a multiplicity of bitter tastants. Among these bitter compounds, 3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin (6-methoxymellein), 5-hydroxy-7-methoxy-2-methylchromone (eugenin), 2,4,5-trimethoxybenzaldehyde (gazarin), (Z)-heptadeca-1,9-diene-4,6-diin-3,8-diol (falcarindiol), (Z)-heptadeca-1,9-diene-4,6-diin-3-ol (falcarinol), and (Z)-3-acetoxy-heptadeca-1,9-diene-4,6-diin-8-ol (falcarindiol 3-acetate) are the major contributors. Of these falcarindiol has the strongest contribution to bitterness in the carrot. In a study conducted on range of carrot products, falcarindiol was quantified in fresh carrots, carrots stored for 5 days at 3 °C, and three carrot puree batches showing consumer complaints. The highest bitter scores were associated with the samples with the highest amounts of falcarindiol, in this case, the carrot purees.
Kreutzmann and others (2008) studied the potential bitter compounds in different carrot genotypes and combined this evaluation with sensory analysis in order to identify key compounds likely to be responsible for the bitterness of carrots. Eight carrot genotypes (‘Bolero’, ‘Mello Yello’, ‘Nairobi’, ‘Tornado’, ‘Purple Haze’, ‘Line 1’, ‘Line 2’, and ‘Line 3’) representing extremes in sensory-perceived odour, flavour, and taste were the subject of study. Potential bitter compounds like polyacetylenes, isocoumarins and phenolic acids were quantified in the peel and the corresponding peeled carrot, and their contribution to bitterness in raw carrots was analysed by sensory profiling using multivariate data analysis. Falcarindiol was found to be highly related to bitterness in contrast to falcarinol and other potential bitter compounds which were primarily present in the peel whereas falcarinol was almost evenly distributed in the root.

1.2.7. Antibacterial, antimycobacterial, and antifungal activity

As previously discussed falcarinol and falcarindiol are antifungal compounds, inhibiting spore germination of various fungi in concentrations ranging from 20-200 µg/mL (Garrod et al., 1978; Kemp, 1978; Harding and Heale, 1980, 1981; De Wit et al., 1981; Elgersma et al., 1984; Imoto et al., 1988; Hansen and Boll, 1986; Nitz et al., 1990; Lutomski et al., 1992; Olsson and Svensson, 1996; Christensen, 1998; Christensen and Brandt, 2006). Recent studies have also shown that falcarinol, falcarindiol and related C_{17}-polyacetylenes have antibacterial effects as well as antimycobacterial effects (Kobaisy et al., 1997; Lechner et al., 2004; Chou et al., 2006; Schinkovitz et al., 2008; Meot-Duros et al., 2010). In particular they exhibit antimycobacterial effects towards Mycobacterium spp. including M. aurum, M. fortuitum, and M. tuberculosis (Kobaisy et al., 1997; Schinkovitz et al., 2008), and antibacterial effects towards resistant strains of the Gram-positive bacterium Staphylococcus aureus (Kobaisy et al., 1997; Lechner et al., 2004; Chou et al., 2006). This is interesting as anti-staphylococcal activity and effects against mycoplasma, as well as other antibacterial effects, occurred at approximately 10 µg/mL, i.e., at concentrations non-toxic for humans.
Figure 1.1. Biochemical route of synthesis of falcarinol type polyacetylenes, adapted from Bohlmann et al. (1973) and Hansen and Boll (1986).
Table 1.1. Occurrence of Polyacetylenes in edible parts of plants

<table>
<thead>
<tr>
<th>Family/species</th>
<th>Common name</th>
<th>Plant part used for foods</th>
<th>Primary use</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anethum graveolens L.</td>
<td>Dill</td>
<td>Leaves, seeds</td>
<td>Condiment or flavouring and vegetable</td>
<td>Bohlmann et al (1973), Degen et al (1999)</td>
</tr>
<tr>
<td>Anthriscus cerefolium (L.) Hoffm.</td>
<td>Chervil, salad chervil, French parsley</td>
<td>Leaves, seeds</td>
<td>Condiment or flavouring and vegetable</td>
<td>Degen et al (1999)</td>
</tr>
<tr>
<td>Articium lappa L.</td>
<td>Edible burdock, Gobo</td>
<td>Root</td>
<td>Vegetable</td>
<td></td>
</tr>
<tr>
<td>A. sylvestris Hoffm.</td>
<td>Cow parsley</td>
<td>Leaves</td>
<td>Vegetable</td>
<td>Christensen (unpublished result), Nakano et al. (1998)</td>
</tr>
<tr>
<td>Apium graveolens L. var. Dulce</td>
<td>Celery</td>
<td>Leaves, seeds</td>
<td>Condiment or flavouring and vegetable</td>
<td>Christensen (unpublished result), Avalos et al. (1995)</td>
</tr>
<tr>
<td>Bellis perennis L.</td>
<td>Common daisy</td>
<td>Leaves, Fruit</td>
<td>Condiment or flavouring and vegetable</td>
<td>Avato et al. (1995), Avato et al. (1997)</td>
</tr>
<tr>
<td>Bunium bulbocastanum L.</td>
<td>Great earthnut</td>
<td>Tubers, leaves, flowers</td>
<td>Condiment or flavouring and vegetable</td>
<td>Bohlmann et al (1973)</td>
</tr>
<tr>
<td>Centella asiatica L.</td>
<td>Asiatic or Indian pennywort</td>
<td>Leaves</td>
<td>Vegetable</td>
<td>Christensen (unpublished result), Nakano et al. (1998)</td>
</tr>
<tr>
<td>Chaerophyllum bulbosum L.</td>
<td>Turnip – rooted chervil</td>
<td>Root, leaves</td>
<td>Vegetable</td>
<td>Christensen (unpublished result)</td>
</tr>
<tr>
<td>Chrysanthemum coronarium L.</td>
<td>Garland chrysanthemum, Shungiku (Japanese), Kor tongho (Chinese)</td>
<td>Leaves</td>
<td>Vegetable</td>
<td>Christensen (1992), Bohlmann et al (1979), Tada et al. (1984), Sanz et al. (1990)</td>
</tr>
<tr>
<td>Cichorium endivia L.</td>
<td>Endive, escarole</td>
<td>Leaves</td>
<td>Vegetable</td>
<td>Christensen (unpublished result), Nakano et al. (1998)</td>
</tr>
</tbody>
</table>

<p>| Coriandrum sativum L.           | Coriander, cilantro | Leaves, seed | Condiment or flavouring and vegetable | Christensen (unpublished result), Nakano et al. (1998)                     |</p>
<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Common Name</th>
<th>Part Used</th>
<th>Category</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Crithmum maritimum L.</strong></td>
<td>Samphire, marine fennel</td>
<td>Leaves, root, stem, flower</td>
<td>Vegetable</td>
<td>Cunsolo et al. (1993)</td>
</tr>
<tr>
<td><strong>Cryptotaenia canadensis (L.) DC.</strong></td>
<td>Hornwort, white or wild chervil</td>
<td>Root, leaves, stem, flower</td>
<td>Vegetable</td>
<td>Eckenbach et al. (1999)</td>
</tr>
<tr>
<td><strong>C. intybus L. var. foliosum</strong></td>
<td>Chicory</td>
<td>Leaves, root</td>
<td>Vegetable</td>
<td>Rucker et al. 1991</td>
</tr>
<tr>
<td><strong>C. japonica Hassk.</strong></td>
<td>Japanese hornwort, Mitsuba</td>
<td>Root, leaves, stem</td>
<td>Vegetable</td>
<td>Nakano et al. (1998)</td>
</tr>
<tr>
<td><strong>Cynara scolymus L.</strong></td>
<td>Globe artichoke</td>
<td>Leaves, flower</td>
<td>Vegetable</td>
<td>Bohlmann et al., 1973</td>
</tr>
<tr>
<td><strong>Ferula assa – foetida L.</strong></td>
<td>Asafoetida, giant fennel</td>
<td>Root, seed, shoot</td>
<td>Condiment or flavouring</td>
<td>Bohlmann (1973)</td>
</tr>
<tr>
<td><strong>F. communis L.</strong></td>
<td>Common giant fennel</td>
<td>Leaves, seed</td>
<td>Condiment or flavouring and vegetable</td>
<td>Appendino et al. (1993)</td>
</tr>
<tr>
<td><strong>Foeniculum vulgare Mill.</strong></td>
<td>Fennel</td>
<td>Leaves, seed</td>
<td>Condiment or flavouring and vegetable</td>
<td>Degen et al (1999), Zidorn et al. (2005)</td>
</tr>
<tr>
<td><strong>Helianthus tuberosus L.</strong></td>
<td>Jerusalem Artichoke</td>
<td>Root</td>
<td>Vegetable</td>
<td>Bohlmann et al. 1973</td>
</tr>
<tr>
<td><strong>Heracleum sphondylium L.</strong></td>
<td>Common cow parsnip, hogweed</td>
<td>Leaves, shoot</td>
<td>Vegetable</td>
<td>Degen et al (1999), Christensen (unpublished result)</td>
</tr>
<tr>
<td><strong>Lactuca sativa L.</strong></td>
<td>Lettuce</td>
<td>Leaves</td>
<td>Salad</td>
<td>Bohlmann et al. (1973), Bentley et al. (1969)</td>
</tr>
<tr>
<td><strong>Levisticum officinale Koch.</strong></td>
<td>Lovage, garden lovage</td>
<td>Leaves, seed</td>
<td>Condiment or flavouring and vegetable</td>
<td>Christensen (unpublished result)</td>
</tr>
<tr>
<td><strong>Lycoopersicon esculentum Mill.</strong></td>
<td>Tomato</td>
<td>Fruit</td>
<td>Vegetable</td>
<td>De Wit et al . 1981; Elgerma et al. 1984</td>
</tr>
<tr>
<td><strong>Myrrhis odorata (L.) Scop.</strong></td>
<td>Sweet cicely, sweet chervil</td>
<td>Root, leaves, seed</td>
<td>Condiment or flavouring</td>
<td>Bohlmann et al. (1973)</td>
</tr>
<tr>
<td><strong>Oenanthe javanica (Blume) DC.</strong></td>
<td>Water – dropwort, water celery</td>
<td>Leaves, stem, shoot</td>
<td>Vegetable</td>
<td>Fujita et al. (1995), Christensen et al. (2006)</td>
</tr>
</tbody>
</table>
**Introduction and literature review**

<table>
<thead>
<tr>
<th>Persea Americana Mill.</th>
<th>Avocado Fruit</th>
<th>Fruit</th>
<th>Adikaram et al. 1992</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. crispum</em> (Mill.) Nyman ex A. W. Hill. Var. <em>tuberosum</em></td>
<td>Hamburg parsley, turnip rooted parsley</td>
<td>Root, leaves</td>
<td>Nitz et al. (1990), Zidorn et al. (2005)</td>
</tr>
<tr>
<td><em>Sium sisarum</em> L.</td>
<td>Skirret, chervil</td>
<td>Root</td>
<td>Schulte et al. (1970), Bohlmann et al. (1973)</td>
</tr>
<tr>
<td><em>Solanum melongena</em> L.</td>
<td>Eggplant, aubergine</td>
<td>Fruit</td>
<td>Bohlmann et al. (1961), Bohlmann et al. (1973)</td>
</tr>
<tr>
<td><em>Trachyspermum ammi</em> (L.) Spr.</td>
<td>Ajowan, ajwain</td>
<td>Leaves, seed</td>
<td>Imoto et al. 1988</td>
</tr>
</tbody>
</table>

Adapted from Christensen et al. (2003)

**1.2.8. Anti-inflammatory and anti-platelet-aggregatory effects**

Numerous studies have reported that falcarinol-type polyacetylenes posses anti-inflammatory and anti-platelet-aggregatory effects (Teng et al., 1989; Appendino et al., 1993; Alanko et al., 1994). For falcarinol it has been suggested that this activity is related to an ability to inhibit lipoxygenases and to modulate prostaglandin catabolism by inhibiting the prostaglandin-catabolizing enzyme, 15-hydroxy-prostaglandin dehydrogenase (Kris-Etherton et al., 2002). In a recent study by Metzger et al. (2008), extracts of purple carrots were shown to possess anti-inflammatory activity by decreasing lipopolysaccharide (LPS) induced production of pro-inflammatory cytokines (IL-6, TNF-α) and nitric oxide (NO) in macrophage cells at concentrations around 10 μg/mL. Purple carrots have very high concentrations of anthocyanins and other polyphenols, which are known to possess anti-inflammatory effects. However, bioassay-guided fractionation of purple carrot extracts revealed that the main anti-inflammatory agents in the extracts were falcarinol, falcarindiol and falcarindiol-3-acetate, which suggests that polyacetylenes are primarily responsible for the anti-inflammatory activity of carrots. Falcarinol and falcarindiol are also strong inhibitors of lipoxygenases (LOX) (5-, 12- and 15-LOX) involved in tumour-progression and atherosclerosis (Alanko et al., 1994; Liu et al., 1998; Schneider and Bucar, 2005 a, b; Prior et al., 2007). Furthermore, falcarindiol is
an effective inhibitor of cyclooxygenases (COX), in particular COX-1, whereas the anti-COX activity of falcarinol does not seem to be pronounced (Alanko et al., 1994; Prior et al., 2007).

Polyacetylenes of the falcarinol-type have also shown to exhibit anti platelet-aggregating activity (Teng et al., 1989; Kuo et al., 1990; Appendino et al., 1993; Alanko et al., 1994). The isolation of falcarinol type polyacetylene from Panax ginseng and from Gymnaster koraiensis with an inhibiting effect on ACAT (Cholestrol acyltransferase) demonstrated that falcarinol type polyacetylenes may aid in the prevention of cardiovascular diseases (Rho et al., 1999). The protective effects of falcarinol-type polyacetylenes against the development of cardiovascular diseases are probably related to their anti-inflammatory activity, and in particular their ability to inhibit certain LOXs that are responsible for the production of thromboxanes, such as thromboxane B$_2$ (Teng et al., 1989; Kuo et al., 1990; Appendino et al., 1993; Alanko et al., 1994). In addition, it has been suggested that the anti-platelet-aggregating activity of falcarinol is related to its ability to modulate prostaglandin catabolism by inhibiting the prostaglandin-catabolizing enzyme 15-hydroxy-prostaglandin dehydrogenase (Fujimoto et al., 1998). The anti-inflammatory and anti-platelet-aggregating activity of polyacetylenes of the falcarinol type are most likely due to their ability to react with nucleophiles such as COXs, LOXs as well as NF-κB at a critical nucleophilic site in this transcription factor.

1.2.9. Cytotoxicity and anticancer effect

The presence of reactive chemicals groups in falcarinol-type polyacetylenes, e.g. triple bonds, that can stabilise radicals and/or carbocations formed at adjacent positions in the molecules, as well as their relatively high levels of toxicity, led to a number of studies on the cytotoxic and anticancer potential of polyacetylenes. The potential anticancer activity of falcarinol-type polyacetylenes was first discovered by the investigation of lipophilic root extracts from Panax ginseng C. A. Meyer (Araliaceae), which led to the isolation and identification of several falcarinol type polyacetylenes of which falcarinol, panaxydol and panaxytriol were found to be the most cytotoxic (Shim et al., 1983; Fujimoto et al., 1988; Ahn et al., 1988; Matsunaga et al., 1989; Matsunaga et al., 1990; Christensen, 1998; Christensen and Brandt, 2006; Christensen et al., 2008; Siddiq et al., 2008).
Kobak-Larsen and others (2005) investigated the anti-cancer properties of falcarinol using a rat model for colon cancer induced by injection of the carcinogen azoxymethane in the inbred rat strain BDX, falcarinol treatments showed a significant tendency to reduce the number of (pre)cancerous lesions (aberrant crypt foci) in physiologically relevant concentrations, i.e., in amounts corresponding to a daily human consumption of 400–600 g fresh weight of carrot (Kobak-Larsen et al., 2005). Matsunaga and coworkers (1990) also reported that falcarinol was, cytotoxic to human gastric adenocarcinoma cells at low concentrations (ED50 of 0.11 mM).

Furthermore falcarinol, panaxydol and panaxytriol have been found to be highly cytotoxic to other cell lines, such as leukemia (L-1210), mouse melanoma (B-16), and mouse fibroblast-derived tumor cells (L-929) (Ahn et al., 1988; Matsunaga et al., 1989, 1990). The most potent toxic effect was observed for MK-1 cells with ED50 values of 0.027 µg/mL, 0.016 µg/mL, and 0.171 µg/mL for falcarinol, panaxydol and panaxytriol, respectively (Matsunaga et al., 1990). In the same study, these polyacetylenes were also shown to inhibit the growth of normal cells in culture, such as human fibroblasts (MRC-5), with ED50 against normal cells being around 20 times higher when compared to the tested cancer cells. This observation is in contrast with a recent study by Purup et al. (2009), who investigated the differential effects of falcarinol and related C17-polyacetylenes on human intestinal epithelial cells of cancer (Caco-2) and normal (FHs 74 Int.) origin, and found that the growth inhibitory effects on normal and cancer cells were almost the same for all investigated polyacetylenes.

Falcarindiol also possesses cytotoxic (Cunsolo et al., 1993; Bernart et al., 1996; Fujioka et al., 1999; Zidorn et al., 2005; Purup et al., 2009) and anti-mutagenic (Miyazawa et al., 1996) activity in vitro, although it appears to be less bioactive than falcarinol. Falcarindiol-8-methyl ether and panaxydiol isolated from celeriac, parsley and carrots are examples of additional polyacetylenes from food plants that exhibit cytotoxic effects on human cancer and leukaemia cell lines (Ahn et al., 1988; Zidorn et al., 2005).

**1.2.9.1. Possible Mechanism of action**

The mechanisms for the cytotoxic activity and potential anticancer effects of falcarinol-type polyacetylenes is still not known but is most likely related to their alkylating properties and hence the presence of a hydroxyl group at C-3. Evidence
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for this can be found in a recent study by Purup et al. (2009) who studied the inhibitory effect of falcarnol and its oxidized form, falcarnone, on human intestinal cell proliferation [Caco-2 and FHs 74 normal intestinal epithelial cells (Int. cells)]. The authors showed that falcarnol significantly inhibited the proliferation of Caco-2 cells at 2.5 μg/mL ($p < 0.01$) and in FHs 74 Int. cells at 5 μg/mL ($p < 0.01$) whereas falcarnone only inhibited proliferation in Caco-2 cells at 20 μg/mL ($p < 0.001$) and only tended to decrease proliferation in FHs 74 Int. cells at this concentration ($p < 0.10$). Furthermore, the 20 μg/mL dose of falcarnone only caused 40-45% reduction in cell proliferation of both normal and cancer cells. These results demonstrate that falcarnone is a much less potent inhibitor of cell proliferation in intestinal cells of both normal and cancerous origin and strongly support the hypothesis that the mode of action of falcarnol-type polyacetylenes is due to their ability to lose water through the hydroxyl group at C-3, thereby resulting in the formation of reactive carbocations (Kobaek et al., 2005; Christensen et al., 2006; Christensen, 2009; Purup et al., 2009). This chemical property of falcarnol-type polyacetylenes indicates that these compounds may affect the potential anticancer activity of each other in an antagonistic and/or synergistic manner. Purup et al. (2009) investigated this hypothesis and found a synergistic response for the inhibitory effect of cell proliferation in both Caco-2 and FHs 74 Int. cells when falcarindiol was combined with falcarnol at concentrations of 1, 5 and 10 times the concentration of falcarnol. These results demonstrate that falcarindiol in low doses can have very potent inhibitory effects on intestinal cell proliferation when used in combination with low doses of falcarnol. Synergistic interactions could therefore be an important factor in relation to the anticancer activity of falcarnol-type polyacetylenes, although this activity clearly depends on the concentration and the ratio of the compounds. Furthermore, the results demonstrate that the bioactivity of falcarnol-type polyacetylenes including synergistic effects is strongly related to their ability to generate resonance stabilized carbocations.

1.3. Extraction and purification of polyacetylenes from natural sources

Falcarnol-type polyacetylenes are known to be highly unstable and are not commercially available in a pure form. Therefore proper method development for their extraction, purification, isolation and characterization from the natural sources is essential. Different methods employed for the extraction of falcarnol-type
polyacetylenes include, but are not restricted to solid-liquid extraction, solid phase extraction using column/flash chromatography, preparatory HPLC with NMR and mass spectrometry used for characterization and structure elucidation.

In particular, chromatographic methods for the isolation of polyacetylenes of the falcarinol-type from plant extracts including a combination of column chromatography (CC) and preparative and semi-preparative high-performance liquid chromatography (HPLC) have been developed allowing isolation of relatively large amounts of these polyacetylenes for the use in preclinical and in clinical trials. Qualitative and quantitative chromatographic methods described for these secondary metabolites, include analytical HPLC combined with UV-detection (Hadacek et al., 1988; Avalos et al., 1995; Wittstock et al., 1995; Zidorn et al., 2002; Kidmose et al., 2004; Stahl and Sties, 2005; Zidorn et al., 2005) and capillary gas chromatographic techniques with flame ionisation and/or mass spectrometric detection (Nitz et al., 1990; Czepa and Hofmann, 2003, 2004; Santos et al., 2005). A method for the quantification of falcarinol and related polyacetylenes in plasma samples using liquid chromatography combined with mass spectrometry (LC-MS/MS) has also been reported (Hansen-Moller et al., 2002; Haraldsdottir et al., 2002a,b,c; Brandt et al., 2004).

1.3.1. Extraction

Most polyacetylenes, including polyacetylenes of the falcarinol-type, are thermally unstable and may undergo photodecomposition if exposed to daylight (Bohllmann et al. 1973). As a consequence, polyacetylenes are usually extracted in low light using relatively low temperatures. Owing to their lipophilic nature, falcarinol-type polyacetylenes are usually extracted from fresh or dried plant material using organic solvents such as ethyl acetate, diethyl ether, methylene chloride or methanol. Sometimes, the extracts are first subjected to bulk chromatography to obtain fractions with different polarity but this is normally not necessary when focusing on lipophilic polyacetylenes (Christensen and Brandt, 2006). In general, polyacetylenes are extracted using simple solid-liquid extraction; however, a new method using pressurized liquid extraction has also been reported (Pferschy-Wenzig et al., 2009) which have been used extensively to extract bioactives from other sources in a high throughput and efficient manner.
1.3.2. Purification

Falcarinol and related polyacetylenes have been separated in extracts by repeated column chromatography (CC) and/or thin-layer chromatography (TLC) on silica gel (Lam et al., 1990; Wittstock et al., 1995; Miyazawa et al., 1996; Hansen et al., 2003; Kidmose et al., 2004) or by using a combination of silica gel CC and gel permeation CC on Sephadex LH-20 (Bernart et al., 1996; Zidorn et al., 2005). For silica gel CC a gradient consisting of different proportions of n-hexane or petrol ether and ethyl acetate or diethyl ether has been used to separate polyacetylenes of the falcarinol-type (Wittstock et al., 1995; Miyazawa et al., 1996; Czepa et al., 2003; Hansen et al., 2003; Kidmose et al., 2004) whereas a mixture of CH\textsubscript{2}Cl\textsubscript{2}/acetone [85:15 (v/v)] has been used to separate these compounds on Sephadex LH-20 CC (Zidorn et al., 2005). Novel techniques such as multilayer coil countercurrent chromatography (MLCCC) have also been used for the isolation of polyacetylenes of the falcarinol-type (Nitz et al., 1990). Techniques such as CC, TLC and MLCCC may be very time-consuming especially if large scale isolation of these compounds from plant material is required and in some cases it may be very difficult to separate the polyacetylenes from other compounds in the extract using these methods. However these problems can be overcome by using preparative and/or semi-preparative HPLC as a final purification step. After separation of the polyacetylenes from the extracts by silica gel and/or Sephadex LH-20 CC the polyacetylenes can be isolated from the crude fractions by preparative HPLC techniques (Fujimoto et al., 1991; Hirakura et al., 1991; Kwon et al., 1991; Bernart et al., 1996; Czepa et al., 2003; Hansen et al., 2003; Kidmose et al., 2004; Kobaek-Larsen et al., 2005). The most widely used HPLC techniques for the isolation of polyacetylenes of the falcarinol-type are preparative and/or semi-preparative reversed phase (RP)-HPLC, with a simple stepwise gradient of aqueous methanol or acetonitrile containing increasing proportions of methanol or acetonitrile (Czepa et al., 2003; Hansen et al., 2003; Kidmose et al., 2004; Kobaek-Larsen et al., 2005). The use of silica and aminopropyl-bonded preparative HPLC columns for large scale isolation of falcarinol and related polyacetylenes has also been described (Fujimoto et al., 1991; Hirakura et al., 1991; Bernart et al., 1996). Preparative or semi-preparative reversed phase columns such as ODS-Hypersil (Zidorn et al., 2002), Ultracarb- ODS (Kwon et al., 1997) and ODS-HG-5 (Hansen et al., 2003; Kidmose et al., 2004; Kobaek-Larsen et al., 2005) has been shown to be very
efficient in separating falcarinol and related polyacetylenes from crude polyacetylene fractions.

1.3.3. Characterisation and quantification of polyacetylenes

1.3.3.1. Analytical HPLC with UV/DAD detection

Separation of polyacetylenes by analytical HPLC has been performed on various types of reversed phase C18 columns such as LiChrospher 100 RP-18 (Hansen et al. 2003, Kidmose et al. 2004), Zorbax Rx-C18 (Zidorn et al., 2002, 2005), Spherisorb 5S ODS (Hadacek, 1988; Wittstock et al., 1995), Econosphere C18 (Avalos et al., 1995), LiChrosorb RP-18 (Washida et al., 2003) and Luna 3 µC18 (2) 100A. A gradient consisting of methanol/water or acetonitrile/water is normally used for the separation. Polyacetylenes of the falcarinol-type have very characteristic UV-spectra due to their conjugated triple bonds and hence they are easily identified in extracts by diode array detection (DAD) (Hadacek et al., 1988; Wittstock et al., 1995). Due to the low number of conjugated unsaturated bonds in their structures the extinction coefficients (ε < 6000 for two triple bonds in conjugation) of these compounds at their characteristic UV-maxima above 230 nm is generally very low (Bohllmann et al., 1973; Hadacek et al., 1988; Wittstock et al., 1995). Instead, by detecting the compounds at 205 nm the UV sensitivity is improved approximately 10-fold and hence the detection of the polyacetylenes is improved. This aspect is especially important in the analysis of extracts from many Apiaceae vegetables where the concentration of these compounds can be relatively low (as little as 5 mg/kg fresh weight; Kidmose et al., 2004; Zidorn et al., 2005). However, depending on the extracts investigated, many other compounds also absorb UV light at 205 nm. Quantification of polyacetylenes by analytical RP-HPLC is performed by using an appropriate internal standard (Zidorn et al., 2005) or using a calibration curve of authentic polyacetylene standards (Hansen et al., 2003; Kidmose et al., 2004).

1.3.3.2. Liquid chromatography mass spectrometry (LC-MS)

Liquid chromatography mass spectrometry (LC-MS) methodologies have been used to characterize purified polyacetylenes and to determine the polyacetylene profile in Apiaceae food plants (Zidorn et al., 2002, 2005). With liquid chromatography-tandem mass spectrometry (LC-MS/MS) or similar techniques it is possible to examine selectively the fragmentation of particular ions. This substantially increases the selectivity and sensitivity of these methods making them very suitable for the
analysis of specific compounds or classes of compounds that occur in very low concentrations in complex matrices such as in biological fluid samples (Hansen-Møller et al., 2002a,b,c; Haraldsdottir et al., 2002; Manners et al., 2003). The most widely employed MS techniques are electron impact (EI) (Jones et al., 1966; Kim et al., 1999; Lim et al., 2001; Czepa and Hoffmann, 2003; Qiang et al., 2003) and/or fast atom bombardment (FAB) mass spectrometry (Kim et al., 1999; Lim et al., 2001; Czepa and Hoffmann, 2003). The following parameters were employed by Zidorn et al. (2005): ionization, positive electrospray ionization (ESI); scanning range m/z 150-600; nebulizer 30 psi; dry gas 8 L/min; dry temp 300 °C; Mass spectrometry (MS) signals observed for compounds falcarinol (FaOH) and falcarindiol (FaDOH) compound: FaOH, 263 [M + H2O + H]+, 245 [M + H]+, and 227 [M - H2O + H]+; FaDOH, 283 [M + Na]+, 265 [M - H2O + Na]+, 247 [M - 2H2O + Na]+, 243 [M - H2O + H]+, and 225 [M - 2H2O + H] respectively. Pferschy-Wenzig et al. (2009) developed a new analytical method for falcarinol from carrot root samples extracted using accelerated solvent extraction (ASE) with ethyl acetate and LC-MS analysis of the extracts. Using this approach, falcarinol was determined by extracting the main ion species generated in the ESI positive mode, m/z 268 [M+H–H2O+MeCN]++, from the full MS chromatogram. Søltoft et al. (2010) used HPLC-MS to elucidate the structure of polyacetylenes in carrots based on accurate mass measurements, isotopic pattern fit (Waters, Milford, MA), and fragmentation patterns. An ultra-performance liquid chromatograph (UPLC) interfaced to a time-of-flight tandem mass spectrometer (TOF-MS) was used for exact mass determinations. The identities were confirmed by comparison with the available in terms of retention times and previously published UV and MS data. MS analysis showed that the identified polyacetylenes had a low abundance of the protonated molecular ions [M + H]+. The [FaOH + H]+ ion was not observed in ESI+ mode, which was the most appropriate mode compared to ESI, where polyacetylenes were non-detectable (Pferschy-Wenzig et al., 2009). Fragments due to the loss of one water molecule from the protonated molecular ion, [M - H2O + H]+, of falcarindiol and falcarindiol-3-acetate had abundances of 75-100% and more modest 21% for falcarinol fragments. In addition, the relative abundance of the falcarindiol [M - H2O - OH]+ ion was 55%, and the relative abundance was 69% for the [M - AcO]+ ion of falcarindiol-3-acetate. Adducts of acetonitrile, [M - H2O + CH3CN + H]+, showed relative abundances of 100% for falcarindiol and falcarinol, in accordance with a
previous study of polyacetylenes using atmospheric pressure chemical ionization (APCI) in positive mode and a Methanol (MeOH) eluent (Zidorn et al., 2002). The latter adduct has also previously been observed as the most abundant ion of falcarinol in ESI+, and the formation of MeCN or MeOH adducts seems to be typical for aliphatic C17-polyacetylenes in positive APCI or ESI ion mode (Pferschy-Wenzig et al., 2009). Because of the low abundance of the protonated molecular ions, the exact masses of the individual polyacetylenes were determined on the basis of the fragment [M - H2O + H]+ and they were within an acceptable range (<5 ppm) from the theoretical values.

1.3.3.3. Capillary gas chromatography (GC) and mass spectrometry (GC–MS)
A small number of studies dealing with the qualitative and quantitative analysis of polyacetylenes of the falcarinol-type by capillary GC–FID and GC–MS have been published (Nitz et al., 1990; Czepa et al., 2003,2004; Santos et al., 2005). Separation of polyacetylenes of the falcarinol-type has been achieved on various types of fused silica gel capillary GC-columns such as DB-5, Rtx-5 and SE-54 and similar phases (Nitz et al., 1990; Czepa et al., 2004; Santos et al., 2005). Quantification of the compounds in extracts is made using an appropriate internal standard or by the use of calibration curves made from authentic polyacetylene standards (Nitz et al., 1990; Czepa et al., 2003,2004; Santos et al., 2005). High resolution gas chromatography was performed with a Type 5890 Series II gas chromatograph (Fisons Instruments, Mainz, Germany) using a 30 m × 0.32 mm DB-5 fused silica capillary, 0.25 mm (J&W Scientific, Fisons) by on-column injection at 40 °C. After 2 min, the temperature of the oven was raised at 10 °C/min to 260 °C and held for 15 min isothermally. The flow of the carrier gas, helium, was 1.8 mL/min. MS analysis was performed with a MAT 95 S (Finnigan, Bremen, Germany) in tandem with the HRGC. Mass chromatography in the electron impact mode (MS/EI) was performed at 70 eV and in the chemical ionization mode (MS/CI) at 115 eV with ammonia as the reactant gas (Czepa et al., 2004).

1.4. The need for processing of vegetables
Vegetables are a perishable food material and, inspite of taking all precautions for post harvest handling, it is impossible to keep all available food in its farm-fresh state thus to make them available for later use they are often processed. The major
emphasis of food processing is preservation or shelf-life extension by preventing undesirable changes in the wholesomeness, nutritive value and sensory qualities. Though processing extends the shelf life of vegetables it affects the stability of bioactive compounds such as polyphenols, carotenoids, vitamins etc (Park, 1987; Soria et al., 2010). Nevertheless there is no or very little information regarding the effect of processing on polyacetylene content.

1.4.1. Minimal Processing
Market sales of ready to use minimally processed fresh vegetables have grown rapidly in recent decades as a result of changes in consumer attitudes. Consumer demands a quality product that is convenient and has fresh like characteristics (Ohlsson, 1994). Minimally processed vegetables may be defined as those subjected to some processing techniques of lesser magnitude than boiling, canning or freezing, but which nevertheless add value to the product before distribution and consumption. (Martin-Diana et al., 2006). The effect of minimal processing of vegetables, on the falcarinol type polyacetylenes haven’t been studied nevertheless there are studies conducted on other lipophilic bioactive compound such as carotenoid found in the same vegetable (carrot); polyacetylene being lipophilic can be assumed to behave similar to carotenoids during minimal processing. Sant’Ana et al. (1998) reported a significant decrease in the levels of α, β and total carotene following shredding of raw carrots this was attributed to exposure of their cut surface to oxygen and light leading to their degradation. A similar result was reported by Ruiz-Cruz and others (2007) regarding the losses of α, and β-carotene following minimal processing (shredding) followed by storage which was attributed to oxidation induced by lipoxygenase-associated free radicals (Klein et al., 1985) and increased exposure of carotenoids to oxygen resulting from exposure to light and oxygen during the processing.

1.4.2. Thermal Processing of vegetables
Vegetables including plants of the Apiaceae family are frequently subjected to various forms of processing to make them more suitable for consumption, as well as more resilient to long term storage. Thermal processing can affect levels of phytochemicals by thermal breakdown and leakage of components or by non-enzymatic factors such as light and oxygen (Davey et al., 2000). The severity of
thermal processing is dependent on the expected shelf life of the end product. For example water immersion processing involves heating of products in water to temperatures of 70°C for 2 min resulting in a cooked and pasteurized product with a shelf life of 5 days at 4°C. Sous-vide processing is aimed at producing products with more fresh like properties and involves heating in vacuum sealed pouches to and end point temperature of 90°C resulting in shelf-life of 21 days at 4°C. Thermal processing also employs techniques such as water immersion cooking / boiling, blanching, vacuum cooking, sterilization etc.

1.4.2.1. Sous-vide (SV) processing

Sous-vide or vacuum-cooked foods are defined as “raw materials or raw materials with intermediate food, that are cooked under controlled conditions of temperature and time inside heat-stable vacuumized pouches” (Schellekens and Martens, 1992). SV products are typically heated at relatively mild temperatures (65-95°C) for a long period of time. After heating the products are quickly cooled and kept in chilled storage (1-4°C) for up to 21 days. There is limited published research and a lack of comparable data on the nutritional aspects of SV cooking. And there is no information regarding the effect of SV processing on polyacetylenes. Though there are some studies done on other lipophilic bioactive compounds such as carotenoids. Patras and other (2009) reported that there was only 4% loss of carotenoids following SV processing and higher retention of carotenoids in SV processed samples than those subjected to water immersion processing, they also retained higher levels of carotenoids during chill storage. However Werlein (1998) reported no changes in the levels of α and β-carotene following SV processing when to raw samples of carrot, though a slight decrease in their levels was noted after 7 days of storage, in the storage period of 21 days. The degradation of carotenoids was attributed to their highly unsaturated chemical structure which has an extensive conjugated double-bond system causing them to be very susceptible to oxidation, isomerization or other thermal-triggered chemical reactions during processing (Sánchez-Moreno et al., 2006).

1.4.2.2. Drying

The preservation of fruits and vegetables through drying dates back many centuries and is based on sun and solar drying techniques. The term drying generally refers to
the removal of moisture from a substance. Air-drying, in particular, is an ancient process used to preserve foods in which the solid to be dried is exposed to a continuously flowing hot stream of air where moisture evaporates. Air-drying offers dehydrated products that can have an extended life of a year but, however, the quality of a conventionally dried product is usually reduced from that of the original foodstuff. No studies has been conducted for polyacetylenes in vegetables during drying, however studies have been carried out on other lipophilic compounds such as α-carotene, β-carotene, lycopene, lutein etc. Reigier et al. (2005) investigated the stability of β-carotene and lycopene during convective air, inert gas drying, microwave vacuum drying, and freeze-drying in carrots. Convection drying at temperatures below 70°C, β-carotene and lycopene contents remained unchanged independent of the drying medium. Freeze-drying did not show any advantage to convection-drying regarding carotenoid retention. Microwave vacuum–drying led to dry products with high carotenoid retention within very short drying times of about 2 h. Better retention of carotenoids was found when the samples were stored in inert gas for a period of up to 6 months compared to those which were stored in air. Koca et al. (2007) studied the effect of drying of carrot with and without blanching. It was found that blanching prior to drying enhanced the retention of β-carotene in dehydrated carrots compared to unblanched dehydrated samples. The high loss of β-carotene in unblanched carrots was attributed to oxidation during drying process with active enzyme system. Frias and others (2010) compared the effect of drying of carrot using convective air drying with blanching as pre-treatment and ultrasound as pre-treatment and both combined as pretreatment. Ultrasound pretreated samples without blanching retained higher amount of β-carotene compared to their blanched or blanched ultrasound counterparts. They inferred that during blanching, the integrity of some carrot cells is disrupted, the tissues are heat-humidified, however during ultrasound pre-treatment, ultrasound waves can affect β-carotene stability, irrespective of ultrasound conditions.

1.4.2. Non-thermal processing
The application of heat treatment is the most common method for stabilizing foods, because of its capacity to destroy microorganisms and inactivate enzymes. However, since application of heat can impair many organoleptic properties and reduce the
contents or bioavailability of some nutrients, there is a growing interest in identifying new non-thermal strategies for food preservation (López et al., 1994).

1.4.2.1. Power Ultra-sound
Power ultrasound has been recognised as a promising processing alternative to conventional thermal treatment in the food industry. Ultrasound has been identified as a potential processing technology to meet the U.S. Food and Drug Administration (FDA) requirement of a 5 log reduction in micro organisms found in liquid foods (Salleh-Mack and Roberts, 2007). The propagation of power ultrasound in a liquid induces bubble cavitation due to pressure changes. These resultant micro bubbles collapse violently in the succeeding compression cycles of propagated ultrasonic waves (Mason et al., 1996). This results in localised high temperatures up to 5,000 K, pressures up to 50,000 KPa, and high shearing effects. Consequently the intense local energy and high pressure bring about a localised pasteurisation effect without causing a significant rise in macro-temperature. The application of ultrasound as a laboratory based technique for assisting extraction from plant material is widely published. The ultrasound extraction trials have demonstrated improvements in extraction yield ranging from 6 to 35% (Vilkhu, Food Science Australia unpublished data). However power ultrasound may be used in combination with other processing techniques to increase its effectiveness. Frias and others (2010) studied the effect of ultrasound assisted hot air drying with respect to blanched hot air drying, on β-carotene in carrot slices. They reported that ultrasound assisted drying caused higher retention of β-carotene (96-98%) compared to blanched hot air drying. They suggested that power ultrasound may be considered as a valuable tool to obtain high nutritive dehydrated carrot products.

1.4.2.2. High Hydrostatic Pressure Processing (HHP)
HHP uses water as a medium to transmit pressures from 300-700 MPa to foods. At ambient temperatures, application of pressures in the range of 400-600 MPa inactivate vegetative micro-organisms and reduce the activity of enzymes resulting in a pasteurised product, which can be stored for a considerable time at 4-6 ºC (Cheftel, 1995). High pressure inactivation of vegetative micro-organisms is caused by membrane damage, protein denaturation and decrease of intracellular pH, suggesting that pressure results in deactivation of membrane-bound enzymes
associated with efflux of protons (Smelt, 1998; Smelt, 2002). The potential HHP for industrial food applications has long been recognised. The method may facilitate retention of food quality attributes such as colour, flavour and nutritional values. The limited effect of HHP (at moderate temperatures) on covalent bonds represents a unique characteristic of this technology (Oey et al., 2008). Therefore, in theory, most of the natural food quality aspects, for example nutritional values, can be maintained during HHP treatment. However little or no information is available on the stability of polyacetylenes during HHP processing and fewer studies have been done on other lipid soluble bioactive compounds such as carotenoids, fat soluble vitamins etc. For example Fernández Garcia and others (2001) reported that HHP treatment did not (or only slightly) affected the carotene stability and during storage at 4°C, the carotenoid content of the pressure treated juices and puree mostly remained constant for 21 days. Pressure treatment appears to influence the extraction yield of carotenoids. Numerous studies have shown an increase in extraction yield of carotenoids due to high pressure processing, e.g., more than 40% increase in carotenoid content of pressurized carrot homogenate (600 MPa/25 ºC/10 min) (De Ancos et al., 2000); 20-43% increase in the carotenoid content of orange juice at 360 MPa in the temperature range between 30 and 60ºC for 2.5, 5 and 15 min (De Ancos et al., 2002); 53.88% increase in the carotenoid content of orange juice after treatment of 400 MPa/40ºC/1 min (Sánchez-Moreno et al., 2005) and a significant increase in the carotenoid content of pressurized (400 MPa/25ºC/15 min) tomato puree compared to either thermal treated or untreated puree (Sánchez-Moreno et al., 2006a). Due to these benefits, HHP technology may be used in combination with other processing technologies to process vegetables while retaining the bioactive compounds.

1.5. Project rationale and key objectives

Though much study has been done on the characterization and bioactivity of falcarinol-type polyacetylenes, key knowledge gaps still exist which need to be investigated, most notably determining the types of falcarinol-type polyacetylenes in the target Apiaceae plant vegetable and the effects of processing technologies on their retention in consumer products.

In addressing these important knowledge gaps, five objectives were identified:

(i) To identify and characterize falcarinol type polyacetylene in carrots,
(ii) To establish the effect of various unit operations on the levels of polyacetylenes in carrots and parsnips,

(iii) To assess the effect of freezing and frozen storage on the levels of polyacetylenes in carrots,

(iv) To determine the effects of thermal processing such as water immersion cooking, sous-vide processing, canning, roasting etc. on vegetables containing falcarinol-type polyacetylenes,

(v) To investigate the effects of novel processing technologies such as power ultrasound and high hydrostatic pressure on the levels of polyacetylenes in carrots.

Chapter one reviews the literature in the area of apiaceae vegetables under three main themes: (1) a general review of polyacetylenes, its occurrence & biosynthesis and health promoting effects; (2) an overview of extraction and purification of polyacetylenes from natural sources; (3) an overview on the need for processing of vegetables. The research conducted to address the knowledge gaps and the results obtained are presented in eight experimental chapters, each of which is organized in the format of a research paper complete with bibliography. The first experimental chapter (Chapter 2) presents work conducted to confirm the structural identity of polyacetylenes in Apiceae vegetable. Characterization was carried out using Negative Ion Tandem Mass Spectrometry.

The majority of processed Apiaceae vegetables are produced by thermal means of which water immersion is the most popular due to the relatively low capital costs involved. Therefore the aim of the second experimental chapter (Chapter 3) was to assess the effect of water immersion thermal processing over a range of temperatures and holding times on levels of polyacetylenes in carrots. In addition, regression modelling are used to develop models which may be of use for understanding the effect of thermal processing on polyacetylene levels in other food matrices.

The third experimental chapter (Chapter 4) focused on the effects of a novel thermal method of processing (sous-vide) on levels of polyacetylenes and colour in parsnip
disks *versus* water immersion cooking immediately after processing and during chill storage.

Whilst polyacetylenes present in more commonly consumed vegetables such as carrots and parsnips are well characterised relatively little is known about the identity of polyacetylenes less popular items such as fennel. Therefore the fourth experimental chapter (Chapter 5) investigates the profile of polyacetylenes in fennel bulb slices and assesses the effect of domestic practices such as boiling and roasting processes on their levels.

The availability of novel non-thermal methods of processing could deliver advantages in the production of some foods in terms of energy costs and retention of heat labile components. Of this ultrasound has emerged as a promising technique for retaining potentially bio-active components. The fifth experimental chapter (Chapter 6) examined the effect of novel processing technology such as ultrasound and compared it with conventional processing technology such as thermal blanching pretreatments on polyacetylenes, carotenoid and colour changes in hot air and freeze dried carrot samples.

The technology to produce high hydrostatic pressure processed products has been around for decades. However, use of this method of processing has only become commonplace relatively recently where it is used to produce high quality minimally processed products with long shelf lives. HHP can be carried out at low temperatures or used in conjunction with heat depending on the expected shelf life of the product. The sixth experimental chapter (Chapter 7) investigated the effect of high pressure-temperature processing on polyacetylene retention as compared to thermal processing (*sous-vide*). In addition, kinetics of polyacetylene degradation as a response to processing was examined.

The practice of selling fresh vegetables in minimally processed forms has become widespread therefore the seventh experimental chapter (Chapter 8) investigates the influence of unit operations such as peeling and cutting on the levels of polyacetylenes in minimally processed carrots and parsnips. In order to best replicate industrial scale condition this trial was carried out in conjunction with an industrial partner on their processing line. The ultimate goal was to provide processors with recommendations for the maximum retention of polyacetylenes in minimally processed carrots and parsnips following processing and chill storage.
Despite the popularity of minimally processed vegetables a large proportion of vegetables such as carrots and parsnips are still sold as frozen goods. Standard industrial protocols for the production of frozen vegetables incorporate a blanching step to inactivate enzymes which might otherwise lead to a reduction in product quality. The eighth experimental chapter (Chapter 9) examines the effect of slow and blast freezing with and without blanching, followed by frozen storage on the polyacetylene content and quality (colour and texture) of carrot discs. In addition, weibull model distribution was used to explain the changes incurred during storage. A general discussion and conclusion (Chapter 10) aimed at examining the impact of thermal processing such as water immersion, sous-vide, roasting, minimal processing and storage had on polyacetylenes in carrots, parsnip and fennel. The effect of novel processing technology such as ultrasound assisted drying and high pressure-temperature processing in comparison to conventional processing technology is also discussed with particular attention to the advantages and disadvantages of novel processing technology in comparison to conventional techniques. A discussion of the impact of unit operation and chill storage on polyacetylene content in carrots and parsnips is also included with a view to providing recommendation for their retention. Finally, this chapter reviews the main conclusions from the research conducted.

1.6. Concluding remarks
As the review of the literature has shown, several important knowledge gaps exists in the current understanding of the types and relevant abundance of falcarinol-type polyacetylenes in vegetables and the effects of different processing technologies on these important phytochemicals. As outlined in Section 1.5, this work seeks to provide a detailed scientific understanding for a number of these knowledge gaps. Chapters 2-9 present the results of this research, which encompass a detailed analysis of the three main polyacetylenes in carrots, the effects of different processing methods on the levels of these compounds in carrots, parsnips and fennel and specific aspects of relevance to industry.

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2. Characterization of Polyacetylenes isolated from Apiaceae vegetables by Negative Ion Tandem Mass Spectrometry

2.1 Introduction

Whilst the presence and nature of polyacetylenes in commonly consumed vegetables such as carrots and parsnips is relatively well established it was necessary before embarking on the present study to confirm their presence and chemical identity in our samples. This is particularly important for polyacetylenes since their importance has only recently become apparent (Brandt et al., 2004; Zidorn et al., 2005; Christensen and Brandt, 2006) it is not possible to purchase commercial authenticated standards of any of the compounds present in these vegetables. Therefore isolation, purification and identification of these compounds from their native source are essential steps for quantification and evaluation of their bioactivity (Christensen and Kreutzmann, 2007; Schmiech et al., 2010; Søltoft et al., 2010). Preliminary identification of the purified polyacetylenes can be achieved by comparing their characteristic ultra violet (UV) spectra with published spectroscopic data as reviewed in Section 1.4.3 (Baranska and Schulz, 2005; Zidorn et al., 2005; Christensen and Brandt, 2006; Christensen and Kreutzmann, 2007). However for definite structural confirmation both nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry analyses of isolated polyacetylenes are necessary.

Mass spectrometry coupled to liquid chromatography (LC-MS) is the commonly used tool for establishing the chemical identity of components in foodstuffs. The technique has been used for identification and quantification of various polyacetylenes in food of plant origin (Jones et al., 1966; Yates and England, 1982; Czepa et al., 2003; Pferschy-Wenzig et al., 2009; Schmiech et al. 2010; Søltoft et al., 2010), marine sponges (Kim et al., 1998, 1999; Lim et al., 2001) and in medicinal plants (Zidorn et al., 2002; Qiang et al., 2011) since the mid 1960’s. The most widely used methods in the investigation of polyacetylenes have been electron impact (EI) (Jones et al., 1966; Kim et al., 1999; Lim et al., 2001; Czepa et al., 2003; Qiang et al., 2011) and/or fast atom bombardment (FAB) mass spectrometry (Kim et al., 1999; Lim et al., 2001; Czepa et al., 2003). Electrospray ionisation (ESI) mass spectrometry, despite exponential growth of its application in health and life sciences
since the 1990’s, is a relatively new analytical tool in food research and industry. To date, few literature reports of ESI-MS coupled to an LC have been made for the characterisation of the polyacetylenes (Zidorn et al., 2002; Moe et al., 2004; Kite et al., 2006; Pferschy-Wenzig et al., 2009; Schmiech et al., 2010; Soltoft et al., 2010; Qiang et al., 2011). The ESI-MS data reported to date have been almost exclusively obtained in the positive ion mode where the sodium or solvent adducts dominated the mass spectra. Only Pferschy-Wenzig et al. (2009) have attempted negative ion mass scanning on pure falcarinol and could not detect it. In this study we present the isolation, purification and characterisation of the polyacetylenes from carrots using the negative ion tandem mass spectrometry.

2.2. Materials and method

2.2.1. Chemicals and sample preparation
Carrots (Daucus carota) & Parsnips (Pastinaca sativa) were purchased from a local wholesaler (Keelings Ltd, Dublin, Ireland), parsnip disks were sous-vide processed and extracted as outlined in section 2.2.2. For identification of polyacetylene degradation products in processed parsnips as outlined in chapter 4. Acetonitrile, ethyl acetate, n-hexane and water were of HPLC grade (99.99%) and obtained from Lennox (Dublin, Ireland). Leucine-enkephalin and deuterated chloroform were supplied by Sigma-Aldrich (Dorset, UK).

2.2.2. Sample extraction and purification
For Processed parsnips samples crude extracts were prepared using acetonitrile as the extracting solvent. These crude extracts were extracted using the optimised accelerated solvent extraction (ASE) method described in section 3.2.4. Crude processed parsnip ASE extracts were cleaned up prior to LC-MS analysis using a Waters Sep Pak C18 syringe SPE cartridges, the cartridges were preconditioned with acetonitrile and water, samples were loaded on to the cartridge and eluted using acetonitrile, with a pre-wash of the cartridge using water.

Extraction and isolation of polyacetylene procedure used was a slight modification of the method described by Hansen et al. (2003), Zidorn et al. (2005), Christensen and Kreuztmann, (2007). For carrots, Five kilograms of the fresh carrots were washed, the tops and bottoms manually removed, and cut in 5 mm disks using a Berkel 800 meat slicer (Berkel Company, Indiana, USA). Since polyacetylenes are
also present in the peel of the root (Baranska and Schulz, 2005), whole carrots were used. The samples were lyophilised using an A6/14 Freeze drier (Frozen in Time Ltd., York, UK) and the resulting powder was mixed with ethyl acetate in a ratio of 1:9 (m/v) and stirred overnight at room temperature. After filtration the extract was concentrated at 40°C using Rotavapor R-200 (Büchi Labortechnik GmbH, Essen, Germany) to a final dry yield of 22 g. This oily residue was then re-constituted in 5 mL hexane and pipetted onto a silica gel column (200 g, 0.040-0.063 mm, Merck, Darmstadt, Germany; diameter 2 cm, height 30 cm) which was preconditioned with hexane.

The stepwise column chromatography gradient was: 0-100% (v/v) ethyl acetate in n-hexane, using a total of 1000 mL (200 mL with 0% ethyl acetate, 200 mL with 25% ethyl acetate, 200 mL with 50% and 200 mL with 100% ethyl acetate) yielding 25 fractions of approximately 40 mL each. Fractions 7-11 contained falcarinol, 13-17 falcarindiol-3-acetate and 19-23 falcarindiol and this was confirmed by LC and mass spectrometry methods. Different fractions of the same compound were combined, concentrated, re-diluted in ethyl acetate, and finally purified using preparative LC. This separation was achieved using a Varian Pro-Star system (Varian Inc, Walnut Creek, USA) consisting of a Pro-Star Polaris 210 pump, a Polaris 510 column oven, a 335 diode array detector, and a Polaris 710 fraction collector. Separation was achieved on a Phenomenex C18(2) Luna column (100 x 21.2 mm, 5µm, 100A) preceded by a Phenomenex guard column of the same phase (Phenomenex, Cheshire, UK). 100% water (solvent A) and 100% acetonitrile (solvent B) were used as mobile phases and the separation was achieved following the gradient described previously by Christensen and Kreutzmann (2007) but with a higher a flow rate of 20.0 mL/min. The peaks eluting at retention time of 27-31, 41-44, 45-50 min for falcarindiol, falcarindiol-3-acetate and falcarinol, respectively, were collected as three separate fractions. The fractions were dried under nitrogen. The levels of falcarinol, falcarindiol and falcarindiol-3-acetate were 3.5 mg/kg, 2.5 mg/kg and 5 mg/kg of fresh weight carrots.

2.2.3. UV and NMR measurements

UV measurements of purified fractions were performed in a Shimadzu UV-1700 spectrophotometer, (Shimadzu instruments manufacturing Co. Ltd., Suzhou, China) using 1mL quartz cuvettes for a UV scan range 190 nm - 380 nm. Nuclear Magnetic
Resonance Spectroscopy (NMR), $^1$H and $^{13}$C experiments of purified fractions were carried out on a Bruker Avance III 500 MHz spectrometer using CDCl$_3$ as solvent. Spectra were referenced with the residual chloroform signal at 7.26 ppm.

2.2.4. Mass spectrometry analysis

Electrospray ionisation mass spectrometry and tandem mass spectrometry experiments were performed on a Q-TOF Premier instrument (Waters Corporation, Micromass MS Technologies, Manchester, UK) interfaced to an Alliance 2695 LC system (Waters Corp., Milford, MA, USA). The specified volume (5 µL) each of the “pure” and “crude” extracts of carrots was injected onto a Phenomenex C$_{18}$ reversed-phase column (100 × 2 mm, 2.5 µm). Chromatographic separation of polyacetylenes was achieved using a gradient solvent system of solvents A (100% water) and B (100% acetonitrile). A step gradient from 20% to 100% B was applied for 35 minutes at 250 µL/min. The column temperature was maintained at 40°C. Electrospray mass spectra were recorded in the positive and negative ionisation modes over an m/z range of m/z 100 to m/z 1000. Stock solutions of 1.0 to 1.2 mg/mL of purified polyacetylenes were prepared in acetonitrile. A 10 µL volume of the stock solution was added to 90 µL of acetonitrile, for mass spectrometric analysis 10 µL of the diluted compounds were mixed with 290 µL of acetonitrile:water (1:1) giving a final concentration of 3-4 ng/µL. The MS/MS data were acquired over m/z range of m/z 50 to m/z 500. The capillary voltage was 3 kV and the de-clustering voltage was 30 V. For the ‘MS$^3$’ experiments, the abundant ions produced by in-source fragmentation in the MS scan were isolated by the quadrupole and subjected to collision induced dissociation (CID). Argon was the collision gas; the collision voltage was set to 2 eV during the ESI-MS and between 10-15 eV for the MS/MS experiments. The source and desolvation temperatures were 120°C and 350°C respectively. Accurate mass measurements of the analytes were carried out using leucine enkephalin (m/z 556.2771 for positive ion and m/z 554.2615 for negative ion) as the lock mass which was introduced into the lockspray source at 5 µL/min from a 250 µL gastight syringe.

2.3. Results and Discussion

2.3.1. Identification and Characterization of polyacetylene in fresh carrots extract
On the basis of their UV, NMR and mass spectra the presence of three polyacetylenes, falcarindiol, falcarindiol-3-acetate and falcarinol, in crude and purified fractions of ethyl acetate extracts from carrot root was confirmed according to the previously published data (Zidorn et al., 2005). The establishment of the identity of each of the polyacetylenes in the purified fractions was of paramount importance as these were used for subsequent studies involving the quantification of these compounds by external calibration in fresh and processed samples.

2.3.2. Identification of degradation products from Sous-vide processed parsnips extract

On the basis of the mass spectra of the unprocessed and sous-vide processed parsnips the degradation products formed were identified, as given in detail in section 4.3.4.

2.3.3. UV and NMR Spectroscopy

Polyacetylenes have characteristic maxima and minima in the 200-300 nm region due to their number of unsaturated bonds which include conjugated triple bonds (Christensen and Brandt, 2006; Kite et al., 2006; Christensen and Kreutzmann, 2007; Qiang et al., 2011). The UV and NMR spectra of the three polyacetylenes agreed with previously reported results (Kite et al., 2006; Hansen and Boll, 1986) (Appendices A1-A6).

2.3.4. Mass Spectrometry

Both positive and negative electrospray ionisation modes were assessed for characterisation of polyacetylenes in vegetable extracts. The total ion chromatograms acquired in the positive ionisation mode from the LC-MS are shown in Figure 2-1. A baseline separation of the three polyacetylenes investigated in this study was achieved within a runtime of 35 minutes. This is an improvement from the previous LC-MS methods reported, in which separation times between 45 and 65 min were required (Zidorn et al., 2002; Kite et al., 2006; Pferschy-Wenzig et al., 2009; Qiang et al., 2011). The LC-MS data were reproducible for all the crude extracts of carrots (Fig. 2-1b). In general, polyacetylenes were more stable to in-source fragmentation in the positive ion mode where [M+Na]^+ ions were most abundant than in the negative ion mode; and this could be the reason for all previously reported information on polyacetylenes being performed by positive ionisation mass spectrometry (Kim et al.,
1999; Lim et al., 2001; Czepa et al., 2003; Zidorn et al., 2005; Kite et al., 2006; Pferschy-Wenzig et al., 2009; Schmiech et al., 2010; Soltoft et al., 2010; Qiang et al.,

![Figure 2.1.](image)

**Figure 2.1.** Total ion chromatograms (TICs) from a C18-reversed phase LC-MS showing falcarindiol (1), falcarindiol-3-acetate (2), and falcarinol (3) in (a) mixture of purified polyacetylenes and (b) the crude extract of carrots.
Figure 2.2. The ESI mass spectra of falcarinol in (a) positive (b) negative ion mode and the measured accurate masses of [M+Na]+ and [M-H]- ions are shown in the inset. The MS/MS spectrum from [M-H]- ions and the ‘MS3’ spectrum from the [M-C₃H₆O-H]- ions are shown in (c) and (d) respectively.
Scheme 2.1. Structure of proposed fragmentation product ions from the MS/MS of [M- H]' and [M-C₃H₆O-H]' (‘MS³’) ions of falcarinol. The loss of hexyl radical from the deprotonated falcarinol is shown by the formation of radical anions m/z 88.0 and m/z 102.1.
2001). Even in the positive ion mode, the falcarinol-type polyacetylenes have shown in-source decomposition with the loss of water molecules being the primary loss (Kim et al., 1999; Lim et al., 2001; Czepa et al., 2003; Zidorn et al., 2005; Kite et al., 2006; Pferschy-Wenzig et al., 2009; Schmiech et al., 2010; Soltoft et al., 2010; Qiang et al., 2011). In the subsequent sections, the CID spectra recorded in negative mode are discussed.

2.3.4.1. Falcarinol

The ESI mass spectrum of falcarinol in the positive ionisation mode showed predominantly the [M+Na]^+ ions at m/z 267.19, [M+K]^+ ions at m/z 283.16 and a very low abundance or absence of [M+H]^+ ions (Fig. 2.2a). Similar observation have been reported on FAB-MS (Kim et al., 1999; Lim et al., 2001) and on ESI-MS (Schmiech et al., 2010; Soltoft et al., 2010; Qiang et al., 2011). Søloft et al. (2010) have found no improvement in the abundance of protonated falcarinol molecules when traces of formic acid (0.1%) were added into the mobile phase. Other studies have shown higher abundance of solvent adducts, such as methanol and acetonitrile, of dehydrated falcarinol or falcarinol-type polyacetylenes in particular in the APCI mode (Zidorn et al., 2005; Kite et al., 2006; Pferschy-Wenzig et al., 2009). In the negative ion mode, the deprotonated falcarinol yielded the major ion at m/z 243.17 and a minor ion at m/z 225.16 indicating the loss of a water molecule (Fig. 2.2b). This observation differed from a previous study where falcarinol was not detected in the negative ion mode (Pferschy-Wenzig et al., 2009). Accurate mass measurement confirmed the elemental composition of the falcarinol, i.e. C_{17}H_{20}O (Figs. 2.2a and 2.2b).

Upon CID, the most abundant product ion was at m/z 187.1, which was also observed at the initial ESI-MS scan (cf. Figs. 2.2b and 2.2c). The fragment ions m/z 187.1 could be accounted for by the loss of butene (C_4H_8) or propenal (C_3H_4O). However, accurate mass measurement on this ion from the MS scan revealed the loss of propenal through cleavage of C3-C4 bond ([C_{14}H_{19}]^- measured m/z 187.1485, calculated m/z 187.1487 Vs m/z 187.1123 for [C_{13}H_{15}O]^+). We speculated a further neutral loss of an ethyl radical from this most abundant fragment ion generated the product ion m/z 158.1, but the ion m/z 158.1 is more likely to be derived directly from the deprotonated molecule by loss of hexyl radical (Figs. 2.2c and 2.2d). The latter proposed fragmentation mechanism was supported by the product ion m/z 102.1.
generated from the ‘MS$^3$’ experiment carried out on the ion peak of m/z 187.1 (Fig. 2.2d, Scheme 2.1). Loss of water with subsequent loss of hexene (or cyclohexane) from the deprotonated falcarinol generated m/z 225.2 and m/z 141.1 respectively. The genesis of the product ion m/z 141.1 from the dehydrated falcarinol was evident from the CID experiment performed on the m/z 225.2 (Appendices A7). The cleavages of C5-C6 and C7-C8 bonds of the precursor ion was localised to the fragment ions m/z 81.0 and 105.0 respectively. The mass difference of 24 Da between these two fragment ions substantiated the presence of two triple bonds in the carbon-chain (C4-C5 and C6-C7). The fragment ions m/z 69.0 was produced due to loss of C1-C2 ethylene followed by a cleavage of C5-C6 bond; this mechanism also explains the formation of product ions m/z 93.0 following the cleavage of C7-C8 (Fig 2.2c, Scheme 2.1).

2.3.4.2. Falcarindiol

Similar to falcarinol, the ESI-mass spectrum of falcarindiol showed [M+Na]$^+$ and [M+K]$^+$ ions at m/z 283.18 and 299.16 respectively in the positive ion mode (Fig. 2.3a). In the negative ion mode, [M-H]$^-$ ions at m/z 259.17 and the loss of butene (C4H8) or propenal (C3H4O) at m/z 203.15 was observed (Fig. 2.3b). Accurate mass measurements using both ionisation modes matched the elemental composition of falcarindiol, i.e. C17H24O2 (Figs. 2.3a and 2.3b). Furthermore, accurate mass measurement on the m/z 203.15 [C16H19O]$^-$ gave m/z 203.1431, calculated m/z 203.1436 Vs m/z 203.1072 for [C13H15O2]$^-$ revealing the loss of propenal and not butene.

When subjected to CID, falcarindiol showed a similar fragmentation pattern to falcarinol with the loss of propenal (C3H4O) resulting the product ion at m/z 203.1; and the cleavages of C5-C6 and C7-C8 bonds producing the minor fragment ions m/z 81.0 and m/z 105.0 respectively (Fig. 2.3c). Similarly the fragment ions m/z 69.0 were also produced via the loss of C1-C2 ethylene followed by a cleavage of C5-C6 bond; this mechanism also explains the formation of product ions m/z 95.0 following the charge-driven cleavage of C7-C8 (Fig 2.3c, Scheme 2.2). The loss of water produced m/z 241.2. Following the loss of propenal, a further neutral loss of ethyl radical for the formation of m/z 174.1 was speculated, but this ion was more likely derived directly from the deprotonated molecule by loss of alkyl radical (Figs. 2.3c and 2.3d). The CID mass spectrum of m/z 203.2 showed the product ions m/z 114.0 and m/z
Figure 2.3. The ESI mass spectra of falcarindiol in (a) positive (b) negative ion mode and the measured accurate masses of [M+Na]+ and [M-H]- ions are shown in the inset. The MS/MS spectrum from [M-H]- ions and the ‘MS3’ spectrum from the [M-C3H6O-H]- ions are shown in (c) and (d) respectively.
Scheme 2.2. Structure of proposed fragmentation product ions from the MS/MS of 
[M-H]⁻ and [M-C₃H₆-O-H]⁻ (‘MS³’) ions of falcariindiol. The loss of alkyl radical from the deprotonated falcariindiol is shown by the formation of radical anion m/z 114.0 following dehydration of [M-C₃H₆-O-H]⁻ ions.
Figure 2.4. The ESI mass spectra of falcarindiol-3-acetate in (a) positive (b) negative ion mode and the measured accurate masses of [M+Na]+ and [M-H]- ions are shown in the inset. The MS/MS spectrum from [M-H]- ions and the ‘MS3’ spectrum from the deacetylated falcarindiol-3-acetate [M-CH₃COOH-H]- ions are shown in (c) and (d) respectively.
Scheme 2.3. Structure of proposed fragmentation product ions from the MS/MS of [M- H] and [M-CH₃COOH-H] (‘MS³’) ions of falcarindiol3-acetate. The loss of hexyl radical from the deprotonated falcarindiol-3-acetate is shown by the formation of radical anion m/z 156.1 following deacetylation.
101.0 that confirmed the loss of hexyl group and a pentyl radical from the deprotonated falcarindiol (Fig. 2.3d, Scheme 2.2). The presence of the major product ion at m/z 107.1 suggested that the hydroxyl group at the C8 position triggered the hydrogenation of the triple bond between the C6 and C7 upon CID (Fig. 2.3c).

2.3.4.3. Falcarindiol-3-Acetate

The ESI mass spectrum of this analyte also showed [M+Na]+ and [M+K]+ ions at m/z 325.19 and 341.18, respectively in the positive ion mode (Fig. 2.4a). Additional, but less abundant ions m/z 285.21 and m/z 243.19 were found in this fraction and assigned to [M-H2O+H]+ and [M-CH3COOH+H]+ respectively. The negative ion mass scan showed an [M-H]− ion at m/z 301.18 along with two other major ions at m/z 241.17 (loss of 60 Da, acetic acid) and m/z 203.14 (loss of propenal and ethenone, i.e. C5H6O2), respectively (Fig 2.4b). The accurate mass measurements on these major ions gave elemental compositions of [C19H25O3]− (measured m/z 301.1805, calculated m/z 301.1804), [C14H19O]− (measured m/z 203.1444, calculated m/z 203.1436 Vs m/z 203.1072 for [C13H13O2]− and [C17H21O]− (measured m/z 241.1618, calculated m/z 241.1592).

The CID mass spectrum of falcarindiol-3-acetate showed the most abundant product ion (m/z 255.2) 46 units lower than the precursor ions indicating the loss of H2O and ethene (Fig. 2.4c). The fragment ion at m/z 283.1 is derived from the loss of H2O alone. As observed in the CID spectrum of falcarindiol, the hydroxyl group at C8 in the falcarindiol-3-acetate also initiated the hydrogenation of the triple bond between C6 and C7 producing the fragment ions m/z 149.0; this observation further corroborates that the adjacent hydroxyl group favoured this reaction (Scheme 2.3). The acetate anion is shown by m/z 59.0 and the [C5H6O2]− ions by m/z 97.0. Cleavage of C5-C6 and C7-C8 bonds produced the fragment ions m/z 123.0 and m/z 147.0, respectively and the mass difference between them identified the presence of two triple bonds in carbon chain (C4-C5 and C6-C7). As expected an additional CID experiment performed on m/z 203.1 from falcarindiol-3-acetate showed identical fragmentation pattern to that obtained from falcarindiol (Fig. 2.4d). The analogue CID experiment on the de-acetylated falcarindiol-3-acetate ion (m/z 241.2) also illustrated the loss of hexyl radical by the product ion m/z 156.1 (Fig 2.4d, Scheme 2.3).

The CID spectra showed that the falcarinol-type polyacetylenes generally underwent fragmentation in two different pathways: one that was initiated with the cleavage of
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C7-C8 bond ($m/z$ 105.0, $m/z$ 105.0/107.1 and $m/z$ 147.0/149.1) found in falcarinol, falcarindiol and falcarindiol-3-acetate respectively (Figs. 2.2c, 2.3c, 2.4c) and the other with the cleavage of C3-C4 bond producing $m/z$ 187.1 from falcarinol, and $m/z$ 203.1 from falcarindiol and falcarindiol-3-acetate. The ‘MS$^3$’ experiments on the $m/z$ 203.1 ion peak in the falcarindiol (and falcarindiol-3-acetate) spectra confirmed the cleavage of the C7-C8 bond (Figs. 2.3d). Furthermore, the ‘MS$^3$’ data established that the loss of alkyl (pentyl and hexyl) radical occurred during the CID of deprotonated falcarinol-type polyacetylenes; this also illustrated that the loss of largest alkyl group (hexyl) in polyacetylenes occurred from the homolytic cleavage of C11-C12 bond (Figs. 2.2d, 2.3d, 2.4d). The observation of a hydrogenation reaction in falcarindiol and falcarindiol-3-acetate, i.e. $m/z$ 105.0/107.1 and $m/z$ 147.0/149.1, suggested that the reaction is charge-assisted, while the $m/z$ 105.0 peak in the CID mass spectrum of falcarinol implied that the charge-remote mechanism operated in the fragmentation of falcarinol.

2.4. Conclusions

This chapter has described the potential of negative ESI-MS and MS/MS for structural determination of polyacetylenes aided by the accurate mass measurements. The major advantages of this approach are: the lack of background interference and the fact that it bypasses MS/MS on sodiated molecular ions which are inherently difficult to fragment under low collision energy and avoids the derivatisation steps which may be required in conventional EI ionisation. Upon CID, all the polyacetylenes produced abundant ions that were useful for structural confirmation particularly via the cleavages of C3-C4, C7-C8 and C11-C12 bonds and therefore this approach has the potentials to be implemented in the MRM-methods for quantitative analysis of falcarinol type polyacetylenes. The transitions $m/z$ 243.2$\rightarrow$187.1 (falcarinol), $m/z$ 259.2$\rightarrow$203.1/107.1 (falcarindiol) and $m/z$ 301.2$\rightarrow$255.2/203.1/149.1 (falcarindiol-3-acetate) are attractive for MRM analysis. As evident from previous quantitative approaches of polyacetylenes, the MRM-methods have been poorly reported thus far and the LC-MS analysis carried out were almost exclusively in positive ion MS mode (Zidorn et al., 2005; Kite et al., 2006; Pferschy-Wenzig et al., 2009; Schmiech et al., 2010; Soltoft et al., 2010; Qiang et al., 2011). Although inclusion of UV-detection in the LC-MS provided high specificity for the falcarinol-
type molecules it required full separation of major polyacetylenes and thus increased
the time of chromatography (Qiang et al., 2011). The MRM-method, when
developed, will significantly reduce the chromatography time since over-lapping of
the analyte peaks does not affect quantification as the components are analysed in
different channels/transitions. In addition, the fragmentation patterns observed could
inform similar studies on other unrelated polyacetylenes from other species such as
marine sponges. Furthermore the optimised LC-MS conditions showed baseline
separation of the common polyacetylenes in crude extracts of carrots within 35
minutes. This is an improvement in comparison to the LC methods described in
literature where separation times between 45 and 95 min were required (Zidorn et al.,
2005; Kite et al., 2006; Christensen and Kreutzmann, 2007; Pferschy-Wenzig et al.,
2009; Qiang et al., 2011).

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3. Modelling the effect of water immersion thermal processing on polyacetylene levels of carrot disks

3.1. Introduction
A wide range of levels has been reported for polyacetylenes in carrot in a number of studies (Czepa and Hofmann, 2004; Kidmose et al., 2004) and a range of factors such as the type of carrot tissue (Baranska et al., 2005), the cultivar (Czepa and Hofmann, 2004; Kidmose et al., 2004) and exposure to water stress (Lund and White, 1990) has been shown to affect native polyacetylene levels in carrot root. Carrots are usually consumed raw or thermally processed and they are added to a wide variety of food products, including canned and frozen foods, dehydrated soups, baby foods and mixed vegetables. Thermal processing has been shown to affect the concentration of some bioactive compounds in vegetables, including polyacetylenes (Hansen et al., 2003; Czepa and Hofmann, 2004; Kidmose et al., 2004; Zidorn et al., 2005). However, in contrast to more well known biologically active components present in carrots, such as polyphenols and carotenoids, less is known about the impact of thermal processing on polyacetylene levels in carrots. In fact the few studies which have examined this important factor have formed part of larger studies assessing other effects, such as variety and agronomic factors (Czepa and Hofmann, 2004; Kidmose et al., 2004).

Thus, the objective of the present study was to assess the effect of water immersion thermal processing over a range of temperatures and holding times on levels of polyacetylenes in carrots. In addition, regression modelling was used to develop models which may be of use in understanding the effect of thermal processing on polyacetylene levels in other food matrices. This technique has been used to successfully model the impact of processing of other bioactive components in foods (Patras et al., 2008). Since instrumental colour is an important quality index, influencing consumer acceptance of processed carrots (Kreutzmann et al., 2007); this parameter was also monitored and modelled.
3.2. Materials and Methods

3.2.1. Chemicals
Acetonitrile (ACN), ethyl acetate and water were of HPLC grade (99.9%) and obtained from Lennox (Dublin, Ireland). Diatomaceous earth was obtained from Dionex (Dionex Camberley, Surrey, UK).

3.2.2. Plant material and sample preparation
Fresh carrots (*Daucus carota*, cv. Nerac) were obtained from a local wholesaler (Donnelly’s, Dublin) and stored at 4°C for a minimum of 24 h prior to analysis. Carrots with no visible damage and of root size 15.5±1.5cm were selected for the experiment. After hand peeling, carrots were sliced into disks (5mm) using a Berkel 800 meat slicer (Berkel company, Indiana, USA), as outlined previously in Chapter 2, Section 2.2.2.

3.2.3. Processing of Carrots
For each time-temperature combination, three batches of carrot disks (∼250 g each) were placed in polyethylene plastic bags (15 × 20 cm, 60 holes/bag; 10 holes of Ø = 1 cm/line, a total of 6 lines/bag). Each bag contained a homogeneous sample of carrot slices of variable diameters and from a range of carrots (random distribution). The samples were then thermally treated in a hot water bath (MGW Lauda M40, Berlin, Germany) at temperatures of 50, 60, 70, 80, 90 and 100°C and holding times 2, 5, 10, 20, 30, 40, 50 and 60 min, respectively. In total, 48 sets (6 temperatures × 8 holding times) of conditions were generated. Core temperature profiles were recorded during the process, using an Ellab E-Val TM9608 data module (Ellab UK Ltd., Norfolk, UK) connected to a portable personal computer. This was achieved by inserting a Standard Ellab SSA-12080-G700-TS temperature probe and an Ellab GKM-13009-C020 packing gland (20 mm) into a 30 mm thick carrot cylinder and recording the heat cycle. Prior to any experiment, all Ellab unit probes were calibrated against a JOFRA (ATC-155B) calibration unit at temperatures of 40 and 100°C and all variations of results associated with the calibration did not exceed ±0.1°C, and T₀ on the graph is the time at which the time lag between the carrot reaching the temperature as that of the water bath and that lag varied with different temperature. Immediately after processing, the samples were
cooled to 4°C and instrumental colour was measured (see Section 3.2.7). Following this step, the samples were blast-frozen (Avon Refrigeration Co., Bristol, UK) at -24°C, freeze-dried (Model A6/14, Frozen in Time Ltd., York, UK) and vacuum-packed using a Vac Star 220 vacuum sealer (Vicquip Ltd., Dublin, Ireland). Freeze-dried samples were individually stored in polyethylene bags at 20°C prior to analysis.

3.2.4. Extraction of polyacetylenes
Extraction of polyacetylenes was performed using an ASE 200 Accelerated Solvent Extraction® automated system (Dionex UK Ltd., Leeds, UK). Prior to extraction, freeze-dried carrot slices (≈250 g fresh weight, ≈27.3 g dry weight) were milled to powder using a blender (Kenwood BL 430, Hertfortshire, UK) and 1 g was filled in the cartridges provided. The remaining volume was filled with diatomaceous earth (Dionex) and a 19.8 mm cellulose filter paper (Dionex) was included at each end of the cartridge. Acetonitrile was used as extracting solvent and the optimized set of conditions found to produce best recoveries were: temperature of 80°C, pressure of 1500 psi, and one extraction cycle. These conditions resulted in negligible levels of polyacetylenes for subsequent extraction on the same cartridge (Appendices A8). The extract was then dried under N₂ at 37°C and the residue re-diluted in 1 mL of acetonitrile, filtered using an Acrodisc LC 25 mm syringe filter (Sigma Chemical Co., St Luis, MO, USA) with a 0.2 µm PVDF membrane and transferred to a 2 mL amber vial prior to reverse phase high performance liquid chromatography (RP-HPLC) analysis.

3.2.5. Determination of Polyacetylene content using RP-HPLC
RP-HPLC analysis was performed using an Agilent 1100 (Agilent Technologies, Wokingham, UK) series HPLC system equipped with UV–Visible detector. Chromatographic conditions used were as described by Zidorn et al. (2005). Separations were achieved on a Luna C₁₈ 100A column (100 x 4.6 mm i.d.; 5 µm particle size) from Phenomenex (Cheshire, UK) at 40°C using the following linear solvent gradient: ACN-H₂O [0-5 min (20:80), 10 min (50:50), 30 min (53:47), 45-50 min (65:35), 55-105 min (100:0)]. The flow rate was 1 mL/min and the injection volume was 10 µL. Runs were monitored using a UV-visible detector at 205 nm. Polyacetylenes, eluting between the 20th and the 50th minute of the run, were identified by peak addition of standard and
quantified using external standard calibration curves. The standards (10-50 µg/mL) used in all cases were isolated from solid-liquid ethyl acetate extraction of freeze-dried carrots and purified, in house, using optimised column chromatography and preparative HPLC methods. The procedure used was a slight modification of the isolation described by Hansen et al. (2003; data not shown). Results were expressed as µg of individual polyacetylene per g of dry weight of carrot (µg/g DW), using the dry matter (DM) formula (Eq. (2) – see Section 3.2.6).

3.2.6. Measurement of instrumental colour

The colour of the carrot disk samples was measured using a Hunter-Lab DP-9000 colour meter (Hunter Associates Laboratory, Virginia, USA) fitted with a 2.5 cm diameter aperture. The instrument was calibrated using the black and white tiles provided. Colour was expressed in Hunter-Lab units \( L^* \) (whiteness/brightness), \( a^* \) (redness/greenness) and \( b^* \) (yellowness/blueness). Three replicate measurements per time-temperature combination were performed and results were averaged. In addition, total colour difference (TCD) and dry matter were calculated using Eqs. (1) and (2) as shown below, where \( L_0, a_0, b_0 \) are the values for raw unprocessed carrots.

\[
\text{TCD} = [(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2]^{1/2} \tag{1}
\]

\[
\%\text{DM (dry matter)} = [1- (X_o -X_i)/X_o] *100 \tag{2}
\]

Where,

\( X_o \) = initial wet weight (wt) of the sample (g)

\( X_i \) = final dry wt. of the sample (g).

3.2.7. Regression modelling

Polynomial regression equations were developed to describe the effects of different temperature treatments and holding times on the content of the three polyacetylenes (FaOH, FaDOH, FaDOAc) and instrumental colour of processed and unprocessed carrots disks. The general form of the quadratic polynomial model regression equation employed in this study is presented in Eq. (3). This equation contains linear terms \( X_1, \)
X₂), quadratic or square terms (X₁²; X₂²) plus an interaction term (X₁X₂) that are also included in the equation. By using this equation, linear, quadratic and interactive effects of the independent variables, temperature (X₁) and holding time (X₂), on the dependent variable (Y) were determined.

\[
Y = \beta_0 + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=1}^{2} \beta_{ij} X_i X_j
\]

where \( Y \) is the predicted response, \( \beta_0 \) the constant (intercept), \( \beta_i \) the linear coefficient, \( \beta_{ii} \) the quadratic coefficient and \( \beta_{ij} \) is the cross product coefficient. \( X_i \) and \( X_j \) are independent variables.

Three observations of different samples for one treatment were carried out. Differences were considered significant at \( p<0.05 \). A two way analysis of variance (ANOVA) was performed using the GenStat v 10.1 software package (Rothamsted, UK). Three-dimensional surface plots were developed using Minitab v.15.0 (Minitab Ltd., Coventry, UK) software.

### 3.2.8. Model validation

The predictive performance of the developed models describing the combined effect of temperature and holding time on independent variables (FaOH, FaDOH, FaDOAc content and instrumental colour) of carrot disks were validated in a separate set of randomly selected conditions. These assessments were carried out by calculating the model performance indices: accuracy factor \([\text{AF}, \text{Eq. (4a)}]\) and bias factor \([\text{BF}, \text{Eq. (4b)}]\) (Ross, 1996; Baranyi et al., 1999; Mataragas et al., 2006; Patras et al., 2008;).

\[
AF = 10^{\frac{\sum_{e} \log(V_p/V_E)}{n_e}}
\]

\[
BF = 10^{\frac{\sum_{e} \log(V_p/V_E)}{n_e}}
\]

Where,

\( n_e \) is the number of experimental data, \( V_E \) is the experimental value and \( V_P \) is the predicted value.
3.3. Results and Discussion

3.3.1. Effect of different thermal treatments and holding time on quality parameters of sliced carrots

Table 3.1 presents the measured parameters of raw unprocessed carrot disks prior to thermal treatment. FaOH, FaDOH and FaDOAc contents of the unprocessed carrots were, on average, 359, 154 and 77 µg/g dry weight (DW), respectively. In other studies on carrots, the FaOH content varied from 20 to 155 µg/g DW (Kidmose et al., 2004), 112.06 µg/g DW (section 8.3.1) but values as high as 245.2 µg/g DW (section 7.3.1), 253.6 µg/g DW (section 9.3.1), 277.5 µg/g DW (section 6.3.1), 310 µg/g DW have also been reported (Czepa and Hofmann, 2004). These values were much higher than those reported for fennel in section 5.3.1; however these values were lower than those for parsnips in section 4.3.1.

The latter authors reported levels of FaDOH and FaDOAc, in a range of carrot cultivars, as varying from 160 to 843 µg/g DW and 70 to 408 µg/g DW, respectively. The values shown here are in closer agreement with those reported by Czepa and Hofmann (2004). The observed differences in polyacetylene content could be attributed to the different cultivar used in the present study, since recent work by Mercier et al. (2006) reported a high degree of variation in polyacetylene content with respect to cultivar.

The effects of holding time and temperature on levels of FaOH, FaDOH and FaDOAc are illustrated in 3D surface plots [Fig. 3.1(a-c)]. A non-significant decrease (p > 0.05) in the levels of FaOH was observed as processing temperature increased from 50 to 100°C with a short holding time of 2-5 min in comparison with the levels in raw unprocessed carrot samples was observed. However, as the holding time was increased, this trend changed such that, at temperatures of 80-100°C and holding times of 20-60 min, a significant increase (p < 0.05) in polyacetylene levels was detected in comparison to raw unprocessed samples. In fact, for carrots heated at 100°C for 60 min, a twofold increase in levels of FaOH was observed compared to the values in the raw and processed samples heated at lower temperatures and shorter holding times (Fig. 3.1). Similar to FaOH levels, FaDOH content decreased significantly (p < 0.05) at lower temperatures (50-60°C)
Table 3.1 Mean levels of three polyacetylenes in fresh carrot (FaOH = falcarinol, FaDOH = falacarindiol and FaDOAc = falcaridiol-3-acetate) and Hunter instrumental colour parameters ($L^*$, $a^*$, $b^*$, Hue Angle, WI & BI).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean value in fresh carrots</th>
</tr>
</thead>
<tbody>
<tr>
<td>FaOH ($\mu g/g$ DW)</td>
<td>$359 \pm 16$</td>
</tr>
<tr>
<td>FaDOH ($\mu g/g$ DW)</td>
<td>$154 \pm 5$</td>
</tr>
<tr>
<td>FaDOAc ($\mu g/g$ DW)</td>
<td>$77 \pm 3$</td>
</tr>
<tr>
<td>$L^*$</td>
<td>$55.84 \pm 2.1$</td>
</tr>
<tr>
<td>$a^*$</td>
<td>$25.15 \pm 3.60$</td>
</tr>
<tr>
<td>$b^*$</td>
<td>$43.26 \pm 4.73$</td>
</tr>
<tr>
<td>Hue</td>
<td>$59.89 \pm 0.83$</td>
</tr>
<tr>
<td>WI (Whiteness Index)</td>
<td>$33.11 \pm 3.13$</td>
</tr>
<tr>
<td>BI (Browning Index)</td>
<td>$161.66 \pm 18.28$</td>
</tr>
</tbody>
</table>

for all holding times (2-60 min) with respect to raw unprocessed samples. At higher temperatures (70-90°C) and longer holding times (5-60 min), a significant increase was found ($p < 0.05$).

A decrease occurred when temperature was raised further, up to 100°C for all holding times in comparison with the values of the samples at 90°C (Fig. 3.1). FaDOAc levels were found to be largely unaffected by processing at all temperatures for short holding times of 2-5 min but, when longer holding times and higher temperatures (60-90°C) were applied, a significant increase was detected in comparison to raw unprocessed samples ($p < 0.05$). The latter was not the case for samples which were processed above 90°C, where a decrease in FaDOAc levels at holding times from 40-60 min was exhibited compared to the levels in samples processed at lower temperatures (50-80°C) (Fig. 3.1).

In general, at higher temperatures, the contents of polyacetylenes increased with increasing processing temperature. This was particularly apparent for levels of FaOH. The levels of FaDOH and FaDOAc decreased at the very high temperature of 100°C. This work indicates that, of the three carrot polyacetylenes studied, falcarinol (FaOH) appears to be more stable than FaDOH and FaDOAc. Kidmose et al. (2004) reported
similar behaviour for FaOH following blanching at 90°C and short holding times. Observed increase in the level of polyacetylenes after heat processing could be a result of increased extractability of the bound polyacetylenes, as heat induces solubilisation of the intercellular cementing pectin, thus, facilitating cell loosening (Waldron, 2004; Roeck et al., 2008). In addition, a general increase in polyacetylene levels at longer holding times was observed. Although this behaviour was unexpected, it could be attributed to the leaching of soluble solids from the carrot matrix (Cazor et al., 2006; Rached, 2009) and retention of polyacetylenes, as these are largely insoluble in water. As a result, the proportion of dry weight polyacetylene levels appears to increase. Further studies are needed to confirm the latter two hypotheses which have been discussed in chapter 6. The behaviour of polyacetylenes with respect to heat processing, especially at lower temperatures, is further complicated by the fact that FaOH is a precursor of both the FaDOH and the FaDOAc and, therefore, in the presence of active enzyme systems, these compounds may be inter-converted (Kidmose et al., 2004). It should also be noted that FaDOH is the main compound responsible for the bitter off-taste in carrots (Czepa and Hofmann, 2004; Kreutzmann et al., 2007). A decrease in the levels of this polyacetylene, due to severe thermal treatment, as in the present study, may increase the acceptability of the product due to decreasing bitterness, since the levels of FaDOH in processed samples are similar to those required for sensory perception of bitterness, as reported by Czepa and Hofmann (2004).

Given that instrumental colour is an important quality index influencing consumer acceptance of processed carrots (Czepa & Hofmann, 2004), this parameter was also monitored and modelled. Colour values for the raw unprocessed carrots were 55.84, 25.15, 43.26 for $L^*$, $a^*$, and $b^*$, respectively. During the water immersion process, it was observed that lightness Hunter values ($L^*$) of carrots were significantly lower for all temperatures and holding times (p < 0.05) in comparison with raw unprocessed samples (Fig. 3.1a). Therefore increasing the severity of heat treatment resulted in darkening of the carrots. Similar results for the darkening of beans and carrots with increasing severity of heat treatment were reported by Leadley et al. (2008) and Patras et al. (2008). $a^*$ Values were also found to decrease significantly (Fig. 3.2b) whereas $b^*$ values
Figure 3.1 3D surface plot showing the effect of temperature and treatment time on polyacetylene levels. a) FaOH- Falcarinol, [Least significant difference (LSD 5%), temperature- 27.92; time-24.02], b) FaDOH- Falcarindiol, [LSD (5%), temperature-16.69; time- 21.35], c) FaDOAc- Falcarindiol-3-acetate, [LSD (5%), temperature- 9.61; time-10.55].
increased (Fig. 3.2c) at higher temperatures and longer holding times, though the latter is not always the case. This phenomenon could be due to the loss of some heat-sensitive carotenes present in carrots during thermal processing (Sulaeman et al., 2001). Differences in perceivable colour can be analytically classified as very distinct [total colour difference (TCD) > 3], distinct (1.5 < TCD < 3) and small (TCD < 1.5) (Patras et al., 2008; Tiwari et al., 2008). In the present study TCD was observed to be very distinct (TCD > 3.0) for all maximum treatment conditions. Changes in colour values with respect to unprocessed samples may be regarded as a negative sensory impact of thermal processing.

3.3.2. Predictive model fitting and validation

A predictive model may only be reliably used in decision making when tested and validated (Jagannath and Tsuchido, 2003). Validation is a vital step that reveals the applicable range of a model and the limits of its performance (Gabriel, 2008). Table 3.2 shows that the predictive variables have non-linear and interactive influences on the response. Thus, utilisation of model Eq. (3) was deemed appropriate for this study. The effects of independent variables on temperature (50-100°C) and holding time (2-60 min) on levels of FaOH, FaDOH, FaDOAc and instrumental colour parameters ($L^*$, $a^*$, $b^*$, TCD) were fitted to second-order polynomial models. From the experimental data and Eq. (3), the second-order response functions were expressed as a function of the independent variables, shown in Eq. (5). For FaOH, FaDOH, FaDOAc, $a^*$ and TCD, the predicted response models were found to fit well with the experimental data with high regression coefficients ($R^2$) of 0.89, 0.76, 0.74, 0.74, 0.69, respectively. However, for $L^*$ and $b^*$, low regression coefficients were observed (0.52, 0.46). Table 3.3 demonstrates that the analyses of variance (ANOVA) for the 3D surface quadratic and linear model, for various parameters, were significant for FaDOH and FaDOAc ($p < 0.0001$). In contrast, quadratic models were found to be insignificant for FaOH (Table 3.3). Thus, only linear and quadratic effects of the independent factors were the major determining conditions that caused significant effects on the response surface.
**Figure 3.2** 3D surface plot showing the effect of temperature and treatment time on colour parameters.

a = $L^*$ [Least significant difference (LSD 5%), temperature-0.819; time-0.953],
b = $a^*$ [Least significant difference (LSD 5%), temperature-1.332; time-1.343],
c = $b^*$ [Least significant difference (LSD 5%), temperature-1.98; time-2.061],
d = $b^*/a^*$, [Least significant difference (LSD 5%), temperature-0.1463; time-0.2121]
Table 3.2 Regression model parameters for the effect of thermal processing on the levels of three polyacetylenes (FaOH, FaDOH and FaDOAc) and instrumental colour parameters ($L^*$, $a^*$, $b^*$) in carrot disks.

<table>
<thead>
<tr>
<th></th>
<th>$FaOH$</th>
<th>$FaDOH$</th>
<th>$FaDOAc$</th>
<th>$L^*$</th>
<th>$a^*$</th>
<th>$b^*$</th>
<th>$b^<em>/a^</em>$</th>
<th>Hue</th>
<th>$\Delta E$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>$\beta_0$</td>
<td>534.391</td>
<td>-530.17</td>
<td>-134.18</td>
<td>58.001</td>
<td>35.2417</td>
<td>58.0303</td>
<td>2.71640</td>
<td>54.7700</td>
</tr>
<tr>
<td>Linear</td>
<td>$\beta_1$</td>
<td>-4.9938</td>
<td>17.2130</td>
<td>5.4809</td>
<td>-0.0678</td>
<td>-0.2885</td>
<td>-0.5794</td>
<td>-0.0372</td>
<td>0.05386</td>
</tr>
<tr>
<td></td>
<td>$\beta_2$</td>
<td>-9.4572</td>
<td>-1.3167</td>
<td>-1.0047</td>
<td>-0.0557</td>
<td>-0.0064</td>
<td>0.01311</td>
<td>-0.0161</td>
<td>0.02720</td>
</tr>
<tr>
<td>Quadratic</td>
<td>$\beta_{11}$</td>
<td>0.02173</td>
<td>-0.1063</td>
<td>-0.0355</td>
<td>-0.0001</td>
<td>0.00124</td>
<td>0.00447</td>
<td>0.00035</td>
<td>0.00071</td>
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<tr>
<td></td>
<td>$\beta_{22}$</td>
<td>-0.0111</td>
<td>-0.0254</td>
<td>-0.0092</td>
<td>0.00085</td>
<td>0.00170</td>
<td>0.00067</td>
<td>-9.5E-05</td>
<td>-0.0015</td>
</tr>
<tr>
<td>Interaction</td>
<td>$\beta_{12}$</td>
<td>0.18565</td>
<td>0.0444</td>
<td>0.0322</td>
<td>-0.0001</td>
<td>-0.0021</td>
<td>-2.7E-05</td>
<td>0.00047</td>
<td>0.00218</td>
</tr>
<tr>
<td>Total Model</td>
<td>$R^2$</td>
<td>89.17</td>
<td>76.14</td>
<td>74.84</td>
<td>52.18</td>
<td>74.35</td>
<td>46.55</td>
<td>84.34</td>
<td>87.09</td>
</tr>
</tbody>
</table>
Table 3.3 ANOVA of the fitted quadratic model for the effect of thermal processing on levels of three polyacetylenes and instrumental colour parameters in carrot disks.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Linear</th>
<th>Quadratic</th>
<th>Cross product</th>
<th>Total model</th>
</tr>
</thead>
<tbody>
<tr>
<td>FaOH</td>
<td>$F$ 30.12</td>
<td>1.44</td>
<td>240.19</td>
<td>148.19</td>
</tr>
<tr>
<td></td>
<td>$p$ &lt;0.0001</td>
<td>0.241</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FaDOH</td>
<td>$F$ 50.73</td>
<td>45.12</td>
<td>21.71</td>
<td>57.43</td>
</tr>
<tr>
<td></td>
<td>$p$ &lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FaDOAc</td>
<td>$F$ 20.47</td>
<td>18.03</td>
<td>40.48</td>
<td>53.53</td>
</tr>
<tr>
<td></td>
<td>$p$ &lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>L*</td>
<td>$F$ 0.84</td>
<td>1.21</td>
<td>0.08</td>
<td>19.64</td>
</tr>
<tr>
<td></td>
<td>$p$ 0.434</td>
<td>0.302</td>
<td>0.781</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>a*</td>
<td>$F$ 3.82</td>
<td>4.99</td>
<td>14.01</td>
<td>52.18</td>
</tr>
<tr>
<td></td>
<td>$p$ 0.026</td>
<td>0.009</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>b*</td>
<td>$F$ 9.39</td>
<td>13.03</td>
<td>0.00</td>
<td>15.68</td>
</tr>
<tr>
<td></td>
<td>$p$ &lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.971</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>b*/a*</td>
<td>$F$ 5.09</td>
<td>7.78</td>
<td>38.41</td>
<td>96.95</td>
</tr>
<tr>
<td></td>
<td>$p$ 0.008</td>
<td>0.001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hue</td>
<td>$F$ 0.27</td>
<td>4.17</td>
<td>18.61</td>
<td>121.40</td>
</tr>
<tr>
<td></td>
<td>$p$ 0.761</td>
<td>0.019</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ΔE</td>
<td>$F$ 0.82</td>
<td>0.91</td>
<td>12.85</td>
<td>40.69</td>
</tr>
<tr>
<td></td>
<td>$p$ 0.4460</td>
<td>0.4050</td>
<td>0.0010</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>WI</td>
<td>$F$ 11.71</td>
<td>16.89</td>
<td>0.41</td>
<td>20.8</td>
</tr>
<tr>
<td></td>
<td>$p$ &lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.5260</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BI</td>
<td>$F$ 12.79</td>
<td>18.74</td>
<td>0.17</td>
<td>29.20</td>
</tr>
<tr>
<td></td>
<td>$p$ &lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.6810</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>
Table 3.4 Bias (BF) and accuracy factors (AF) for the responses studied in the regression modelling (n: number of observations – different set of temperature and time conditions).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>(n)</th>
<th>Bias factor</th>
<th>Accuracy factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>FaOH</td>
<td>48</td>
<td>0.9525</td>
<td>1.1349</td>
</tr>
<tr>
<td>FaDOH</td>
<td>48</td>
<td>1.2334</td>
<td>1.5383</td>
</tr>
<tr>
<td>FaDOAc</td>
<td>48</td>
<td>0.9147</td>
<td>1.2005</td>
</tr>
<tr>
<td>(L^*)</td>
<td>48</td>
<td>0.9964</td>
<td>1.0431</td>
</tr>
<tr>
<td>(a^*)</td>
<td>48</td>
<td>1.0286</td>
<td>1.2025</td>
</tr>
<tr>
<td>(b^*)</td>
<td>48</td>
<td>0.9421</td>
<td>1.1150</td>
</tr>
<tr>
<td>(b/a)</td>
<td>48</td>
<td>0.9224</td>
<td>1.1243</td>
</tr>
<tr>
<td>Hue</td>
<td>48</td>
<td>0.9824</td>
<td>1.0321</td>
</tr>
<tr>
<td>(\Delta E)</td>
<td>48</td>
<td>0.9712</td>
<td>1.4941</td>
</tr>
<tr>
<td>WI</td>
<td>48</td>
<td>1.0633</td>
<td>1.0991</td>
</tr>
<tr>
<td>BI</td>
<td>48</td>
<td>0.9208</td>
<td>1.1666</td>
</tr>
</tbody>
</table>

The regression coefficients of Eq. (5) are given in Table 3.2.

\[
Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{12}X_1X_2
\]  

(5)

where \(Y_i\) is predicted response for FaOH, FaDOH, FaDOAc, \(L^*\), \(a^*\), \(b^*\), TCD.

For FaOH, FaDOH, FaDOAc, \(a^*\) and TCD, the predicted response models were found to fit well with the experimental data with high regression coefficients (\(R^2\)) of 0.89, 0.76, 0.74, 0.74, 0.69, respectively. However, for \(L^*\) and \(b^*\), low regression coefficients were observed (0.52, 0.46). Table 3.3 demonstrates that the analyses of variance (ANOVA) for the 3D surface quadratic and linear model, for various parameters, were significant for FaDOH and FaDOAc (\(p < 0.0001\)). In contrast, quadratic models were found to be insignificant for FaOH (Table 3.3). Thus, only linear and quadratic effects of the independent factors were the major determining conditions that caused significant effects on the response surface.
The factors considered in this study caused a specific effect on the degradation of colour and an increase in polyacetylene levels (Fig. 3.2 a-d). The cross product for all of the models strongly fit the experimental data, except for $b^*$. The Fisher F-test results (very low p-values) demonstrated the high significance of the model and convey that the predictive variables (temperature, holding time) can be used to reliably predict the response variable. This study also dealt with the validation of the developed model using a set of data obtained from additional test runs, exclusive to those performed in the elaboration of the model, as recommended by Ross (1996) and Carrasco et al. (2006). Therefore, separate randomly selected data sets of 60 and 90°C, with holding times from 2 to 60 min, were analysed to determine the accuracy of the predicted model. The experimental data were plotted against the predicted values from Eq. (5) for polynomial models. Predicted values of colour parameters obtained using model equations were in good agreement with the experimental values. The experimental and predicted values closely correlated, as demonstrated by regression coefficient ($R^2$) values of 0.78, 0.83, 0.64, 0.89, 0.82, and 0.94 for FaOH, FaDOH, FaDOAc, $L^*$, $a^*$ and TCD, respectively. Fig. 3.3(a-d) shows the predicted and actual (experimental) values for only FaOH, FaDOH, FaDOAc and $L^*$ parameters. The predicted values were within the 95% confidence interval, as illustrated in Fig. 3.3(a-d). To confirm the adequacy of the fitted models, studentised residuals versus run order were tested and the residuals were observed to be scattered randomly, suggesting that the variances of the original observations were constant for all responses (Appendix A9). The normality assumption was also satisfied as the residual plot approximated to a straight line for all observed responses (Appendix A9). It should be noted that the model parameters used for prediction are applicable only to the experimental conditions employed. The applicability of the models was also quantitatively evaluated by comparing the bias and accuracy factors for each of the parameter Eqs. (4a) and (4b)]. In most cases, as shown in Table 3.4, the accuracy factor (AF) values for the predicted model were close to 1.00, except for FaDOH (1.50), TCD (1.49). The bias factor (BF) values for the predicted models were also close to 1.00, ranging from 0.91 to 1.23 for all the parameters studied. These results indicate that there was a good agreement between predicted and observed values. Ross and coworkers (2000) reported that predictive models should ideally have
an AF = 1.00, indicating a perfect model fit where the predicted and actual response values are equal. However, Ross et al. (2000) and Carrasco et al. (2006) reported that the AF of a fitted model increases by 0.10-0.15 units for each predictive variable in the model. F models, as used in this study, that forecast a response from two predictive variables (temperature, time) may be expected to have AF values ranging from 1.20 to 1.30 (Ross et al., 2000) or an equivalent percentage error range of 20-30%.

Despite some variations, results obtained from the validated predicted model and actual experimental values showed that the established models reliably predicted FaOH, FaDOH, FaDOAc, L*, a*, b* and TCD. The predicted values were in close agreement with experimental values and were found to be not significantly different at p > 0.05 using a paired t-test. Therefore, the predictive performance of the established model may be considered acceptable under the experimental conditions employed.
Figure 3.3 Predicted and actual (experimental values) for (a) FaOH, (b) FaDOH
Figure 3.3 Predicted and actual (experimental values) for (c) FaDOAc, (d) $L^*$
3.4. Conclusions

This study dealt with the effect of water immersion thermal processing of carrot disks on the levels of polyacetylenes and instrumental colour. Predictive regression models were developed for the estimation of FaOH, FaDOH, FaDOAc contents and Hunter colour parameters with respect to thermal processing. Processing temperature and holding time had significant effects on the levels of polyacetylenes in carrot disks. In general, at higher temperatures, the levels of polyacetylenes increased with increasing processing temperature. This was particularly true for levels of FaOH. The levels of FaDOH and FaDOAc decreased at the very high temperature of 100°C however if the calculation would have been done on fresh weight basis taking into account the leaching of soluble solids there will be no increase in the polyacetylene level, this phenomena has been discussed more in chapter 5.

This work indicates that, of the three carrot polyacetylenes studied, falcarniol (FaOH) appears to be the most stable. Results of the model validation showed that the developed model had acceptable predictive performance, as assessed by mathematical and graphical model performance indices. The coefficient of determinations \( R^2 \) for predicted FaOH, FaDOH, FaDOAc, and instrumental colour parameters showed good correlation with the experimental data at the 95% confidence level and indicated that second-order polynomial regression models could be employed to predict the levels of the three polyacetylenes (FaOH, FaDOH, FaDOAc) and instrumental colour in carrot disks after thermal processing.

3.5. References


DrLange (1999). Fundamentals of colourimetry–application report No. 10e. DrLange, USA.


4. Influence of Sous-Vide and Water Immersion Processing on Polyacetylene Content and Instrumental Colour of Parsnip (Pastinaca sativa) Disks

4.1. Introduction

Parsnip (Pastinaca sativa) is a root vegetable of the family Umbelliferae that has been cultivated since Roman times for its long, fleshy, edible root. Parsnips can be eaten raw, boiled, roasted, fried or used in stews, soups, casseroles and they are an important part of the diet in the UK and Ireland (Britannica Concise Encyclopedia, 2006). Recent studies have indicated that a group of C_{17} acetylenes of the falcarinol type present in members of the Apiaceae and Araliaceae families have cytotoxicity against human cancer cells (Hansen et al., 2003; Kobaek-Larsen et al., 2005; Metzger et al., 2008). In common with other Apiaceae species the main role of polyacetylenes in parsnips is to prevent fungal infections in the plant (Olsson and Svensson, 1996) and they also impart a bitter taste at high concentrations (Kreutzmann et al., 2008). A previous study have shown that parsnip roots contain two polyacetylenes namely, falcarinol and falcarindiol (Zidorn et al., 2005). Parsnips are normally thermally processed prior to consumption and this may effect the final polyacetylene content in the product, however to date, there have been no reports on the effect of thermal processing on parsnip polyacetylene content, though similar results were observed for carrots (Hansen et al., 2003).

Thermal processing of parsnips usually involves either water immersion (including boiling) or oven roasting. The increase in consumer demand for minimally processed, refrigerated convenience foods with characteristics closer to that of the fresh products has led to the development of technologies which can help retain potential health-promoting compounds in thermally processed foods. Refinement of vacuum packaging, pasteurization and chilled storage of various food products has given rise to a process known variously as sous vide, ‘cuisine en papillote sous vide’, ‘cuisson sous vide’ and sous vide cook-chill (Church and Parsons, 1993). Sous vide (SV) processing, as an alternative to boiling or water immersion (WI) treatments, uses milder temperatures (≈90°C) than sterilization to heat process products in vacuum sealed pouches. This technology may be an effective strategy to minimize losses of thermally unstable compounds during processing (Schellekens and Martens, 1992), while also extending the shelf-life (20 days at 4°C). SV products are stored in vacuum packed pouches following heating
which may also serve to protect compounds susceptible to oxidation (Creed, 1995). Additionally, during processing and storage, colour, as part of the sensory characteristics, may change in parsnips. As previously stated, the effect of a number of parameters in the polyacetylene levels and colour of SV and WI processed parsnips has not been studied, hence the potential outcomes could lead to recommendations for processing and storage practices in households and industries aimed at minimising losses of polyacetylenes in parsnips. Thus, the aim of the present study is to compare the effect of SV processing on levels of polyacetylenes and colour in parsnip disks in comparison to WI cooking immediately after processing and during chill storage.

4.2. Materials and methods

4.2.1. Chemicals
HPLC grade Acetonitrile (ACN), and water were purchased from Fischer Scientific (Dublin, Ireland), Diatomaceous earth for Accelerated Solvent Extraction (ASE) was obtained from Dionex (Surrey, UK).

4.2.2. Sample preparation
Parsnips (Pastinaca sativa cv. Gladiator) were obtained from a local wholesaler (Sea View Ltd., Dublin, Ireland). Parsnip root samples with no visible damage were selected for the experiment. And they were minimally processed ie. they were washed, hand peeled and then sliced in to disks (5 mm) using a Berkel 800 meat slicer (Berkel company, Indiana, USA). Only parsnips with root diameters in the range 60-85 mm were selected for the study. Since peeling and slicing of was carried out on all samples, parsnips which had undergone only these operations and no subsequent thermal processing are hereafter referred to as minimally processed samples.

4.2.3. Sous-vide (SV) processing of parsnip disks
Three batches (1.5 kg each) of peeled and sliced parsnips were subjected to blanching in water at 95°C for 90 s to inactivate enzymes. Samples (250g) were vacuum packed in 20 cm x 30 cm polyethylene bags (thickness – 75 μm, gas permeability of 2.7 g/m² d, Packex Industries Ltd., Wicklow, Ireland) and heated in a retort unit (Barriquand Steriflow, Roanne, France) to a process equivalent of 90 °C for 10 min (P90 ≥ 10). This heat cycle approximated the minimum recommended SV
pasteurization required to inactivate psychrotrophic *Clostridium botulinum* spores and give a chilled shelf-life of 21 days (Gaze and Brown, 1990; Gould, 1990). Sample core temperature profiles and $P_0$ values were recorded during the process using an Ellab E-Val TM TM9608 data module (Ellab Ltd., Norfolk, UK). As outlined previously in Chapter 3, Section 3.2.3, a standard Ellab SSA-12080-G700-TS temperature probe was inserted through an Ellab GKM-13009-C020 packing gland (20 mm) into a 30 mm thick parsnip cylinder to monitor the cook cycle. An Ellab SSR-60020-G700-TS water probe was used to record the cook cycle water temperature. Temperature was recorded every 20 s and the accuracy of temperature measurements was ± 0.1°C. Prior to any experiment, all Ellab unit probes were calibrated against a JOFRA (ATC-155B) calibration unit at temperatures from 40 and 100°C. All results associated with the calibration did not exceed ±0.1°C. Following processing, samples were stored vacuum packed at 4°C for 20 days. Samples (±250 g) were taken after 0, 5, 10, 15 and 20 days of storage. All samples (including minimally processed ones) were blast frozen (Avon Refrigeration Co., Bristol, UK), freeze dried (Model A6/14, Frozen in Time Ltd., York, UK), vacuum packed using a Vac Star S220 vacuum sealer (Vicquip Ltd., Dublin, Ireland) and stored in polythene bags at -20°C.

### 4.2.4. Water immersion (WI) cooking of parsnips

Three sets (250 g each) of raw peeled and sliced parsnips were subjected to boiling in water until a core temperature of 70°C was achieved. They were held at this temperature until they had reached a time-temperature ($P_{70} \geq 2$ min) equivalent to a six log reduction in numbers of vegetative cells of the target pathogen (*Listeria monocytogenes*). Sample core temperature profiles and $P_0$ values were recorded during the process as described above for SV processing. Following processing, the parsnip samples were stored at 4°C for 5 days. Samples (±250 g) were taken after 0, 1, 3 and 5 days of storage, freeze dried and stored at -20°C prior to extraction.

### 4.2.5. Extraction of polyacetylenes

Extraction of polyacetylenes was performed using method described in section 3.2.4.
4.2.6. Determination of polyacetylenes using RP-HPLC
Separation and quantification of polyacetylenes was carried out by RP-HPLC using the method described in section 3.2.5. And the amount of polyacetylenes were expressed in fresh weight taking into account the leaching effect during blanching and water immersion processing.

\[
\text{Fresh wt. (µg/100g FW) = quantified amount on HPLC (µg/g DW) \times % dry matter for that sample}
\]

4.2.7. Liquid Chromatography/Time-of-Flight Mass Spectrometry
Liquid Chromatography/Time-of-Flight Mass Spectrometry and sample preparation for it was carried out for parsnip extract as described in Section 2.2.2 and 2.2.4.

4.2.8. Measurement of instrumental colour
Instrumental colour was measured using the method described in section 3.2.6. All the measurement was carried in triplicate.

4.2.9. Statistical Analysis and experimental design
Differences were considered significant at p<0.05. Analysis of variance (ANOVA) was performed using the GenStat Release 10.1 (PC/Windows XP). In total 30 treatments were examined: 3 for blanched samples (three replicates); 15 for sous-vide processed samples: five storage time (0, 5, 10, 15, 20 days) \times three replicates; 12 for water immersion cooked samples: 4 storage time (0, 1, 3, 5 days) \times three replicates.

4.3. Results and discussion

4.3.1. Effect of thermal processing on polyacetylene levels of parsnip disks
A typical HPLC chromatogram of a minimally processed, freeze-dried acetonitrile extract from parsnip is illustrated in Figure 4.1. The peaks eluting at 24.74 min and 47.64 min were identified as falcarindiol and falcarinol respectively on the basis of their retention times on HPLC. These polyacetylenes were also identified in parsnips of other varieties grown or sold in Ireland (e.g. Dagger, Javelin, etc – data not shown). The levels of falcarinol and falcarindiol in minimally processed parsnip were found to be 7.42 and 4.90 mg/100 g fresh weight (FW). These levels are significantly lower than those reported by Zidorn et al. (2005) of 29.20 and 105.28
Figure 4.1. Sample HPLC chromatograms of a minimally processed freeze dried parsnip extract *Pastinaca sativa*, cv. Gladiator
mg/100 g FW of the sample for falcarinol and falcarindiol respectively. This is probably due to the fact that they were minimally processed ie. peeled and sliced. Carrot peel has been shown to be particularly rich in polyacetylenes in previous studies (Baranska and Schulz, 2005; Kreutzmann et al., 2008) although similar evidence for parsnips is not available. In the present study, parsnips were peeled and sliced in order to mirror the protocol followed in an industrial setting.

A typical time-temperature profile of WI and SV processed parsnip disks, as monitored using the Ellab electronic temperature logging system, is presented in Figure 4.2. Thermal processing had a significant (p≤0.05) effect on polyacetylene levels in parsnip disks as illustrated in Figure 4.3. The levels of falcarinol for minimally processed, blanched, SV processed (day 0) and WI processed (day 0) were 7.42, 5.37, 5.63 and 6.61 mg/100 g FW. The corresponding values for falcarindiol were 4.90, 3.75, 3.40 and 3.82 mg/100 g FW. In the present study blanching (95°C, 90 s) resulted in a significant reduction in polyacetylenes levels (p<0.05) prior to SV or WI treatment. In fact, a reduction of 27% and 24% was observed in the levels falcarinol and falcarindiol in blanched samples compared to minimally processed disks. This is the first report on the effect of blanching on polyacetylenes in parsnips, however the degree of degradation of falcarinol following blanching is in accordance with 50% loss reported by Hansen et al. (2003) after blanching (3 min, 100°C) for carrots. Kidmose et al. (2004) also reported that blanching of carrots can result in a decrease in falcarindiol and falcarindiol-3-acetate by 26.2% and 30.4% respectively. The authors speculated that this was caused by degradation of heat-sensitive polyacetylenes during blanching and/or conversion of falcarinol to falcarindiol and falcarindiol-3-acetate. Blanching is carried out to help inactivate enzymes which could lead to reductions in product quality, however in the present study it appears to have negative effect on one measure of quality i.e., phytochemical content. In comparison with minimally processed samples, losses in SV processed samples were in the range of 24% and 30% for falcarinol and falcarindiol, respectively. Hansen et al. (2003) reported that boiling for longer periods (3-15 min) caused a further 20% loss of total falcarinol in carrot disks. However, in comparison to blanched samples no significant additional decrease in falcarinol or falcarindiol occurred following SV processing (p>0.05). This indicates that blanching was the major cause of polyacetylene loss in parsnips rather than the
Figure 4.2. Time-Temperature profile for parsnip disks during *sous-vide* (SV) processing and water immersion (WI) processing.
Figure 4.3. Effect of sous-vide (SV) and water immersion (WI) processing on levels of polyacetylenes in parsnips (*Pastinaca sativa*, cv. Gladiator) disks at day 0 (immediately after thermal processing).
SV processing itself. On average, WI processing resulted in a slight reduction in, loss of falcarindiol as compared to the loss occurred due to SV processing; however, the difference was not significant (p>0.05). Falcarinol levels for WI processed samples were significantly higher than for SV processed samples (p<0.05). However, it should be noted that SV processed samples had undergone a blanching step (95°C, 90 s) prior to SV processing which was not the case for WI samples. The use of blanching prior to SV processing is highly likely as processors may buy-in prepared (e.g. blanched) product for convenience and to avoid washing/peeling/slicing operations associated with raw carrots and to avoid any enzymatic discolouration due to time lag between minimal processing and sousvide processing (Tansey et al., 2005). To the best of our knowledge, to date, no other reports on the effect of SV processing on polyacetylenes have been published. Nonetheless, the technique has been examined for other potential bio-active compounds. For example, Patras et al. (2009) reported that the SV processing of carrot disks resulted in higher losses of polyphenols compared to WI processed samples. The authors commented that this may be due to the fact that SV processed samples received a more severe treatment in comparison to WI processed samples (90°C for 10 min vs. 70°C for 2 min). This would also be the case in the present study as identical protocols were employed. Stea et al. (2006) reported that SV processed broccoli had low levels of folate which they also attributed to an additional pre-treatment with blanching. Higher losses of falcarindiol also suggest that this compound may be more unstable to heat treatment than falcarinol. A similar trend was found in previous work in this laboratory related to water immersion processing of carrots (see chapter 3 and Rawson et al. 2010a).

4.3.2. Effect of chill storage on polyacetylene levels of parsnip diskss

Vacuum chill (4°C) storage of SV processed parsnips disks (Figure 4.4) resulted in a significant decrease in the level of falcarinol (≈13% loss) after 5 days storage compared to those present in SV processed samples immediately after processing (p<0.05). This decrease continued following prolonged storage resulting in a total loss of ≈ 25% by day 20 when compared to the levels of day 0 (p<0.05). Levels of falcarinol in WI processed samples also decrease during chill storage (Figure 4.5), however the duration of the storage period was shorter for these
samples as determined by the shelf life of the product. Compared to samples immediately after processing (day 0), a WI processed sample at day 5 showed a significant loss (p≤0.05) of ≈ 10% in the levels of falcarinol.

In contrast to falcarinol levels, falcarindiol levels in SV processed samples did not significantly decrease over the 20 day storage period when compared to day 0 samples. On average, losses were of the order ≈ 9.5%; however, the effect was not significant (p>0.05). Aerobic storage, following WI processing, of parsnips disks did result in a significant decrease in the levels of falcarindiol following 5 days chill storage (p≤0.05). In fact, total losses of falcarindiol in these samples were severe (nearly up to 70% when compared to day 0). SV processed samples were stored under vacuum after processing whereas water immersion processed samples were stored in air, therefore the lower stability of falcarindiol in WI samples could be due to oxidative degradation of this compound when stored in air. To date there have been no reports on the stability of polyacetylene compounds during chill storage (4°C) of processed vegetables. Hansen et al. (2003) reported a reduction in falcarinol content in chill-stored raw carrots. However, as those authors suggested, this reduction was probably due to changes in the balance between enzymatic degradation of falcarinol and its biosynthesis and these enzymes would presumably not have been active in the processed samples (Hansen et al., 2003). Additionally, Kidmose et al. (2004) reported that levels of falcarindiol and falcarindiol-3-acetate were reduced in the blanched frozen carrots stored for 4 months because of degradation of heat sensitive polyacetylenes during blanching. Chill storage of thermally processed vegetables has been shown to result in a reduction in the levels of other bioactive compounds. For example, Patras et al. (2009) established that a decrease in levels of polyphenols occurred in SV and WI processed carrot disks during chill storage.

4.3.3. Effect of thermal treatment and storage on instrumental colour of parsnip disks

While the focus of the present study was to monitor the effect of thermal processing on levels of polyacetylenes in parsnip disks we were also interested in the effect of thermal processing on levels of a commonly studied index of quality. Therefore instrumental colour was monitored following thermal processing and during storage.
Hunter colour values for minimal, WI and SV processed and stored parsnip disks are presented in Figures 4.6 and 4.7. Following thermal processing by both methods, $L^*$ values (lightness) of parsnip samples decreased indicating that disks got darker following thermal treatment. In addition, there was a significant decrease in the $L^*$ value from day 0 to day 5 for SV processed samples ($p \leq 0.05$). Further storage of the parsnip disks did not result in changes of the $L^*$ parameter. In WI processed samples, $L^*$ values remained constant during storage until day 5 ($p > 0.05$). To the best of our knowledge no other reports on the effect SV processing on instrumental colour of parsnip disks have been published, although some work has been done on carrot disks. Wierlen (1998) reported that $L^*$ values of carrot disks remained unaffected during storage of SV processed samples. In addition, Araya et al. (2009) reported that $L^*$ values decreased immediately after thermal processing in comparison to minimally processed samples in carrot disks, in SV processed samples $L^*$ values did not change over time, while in WI processed samples $L^*$ diminished during storage.

The values of $a^*$ (+ive = redness, -ive = greenness) increased for SV processed parsnip discs during storage from day 0 to 5 ($p < 0.05$) but no significant change was found thereafter. This indicates that during the first 5 storage days parsnip disks became less green. For WI processed samples there was a significant increase in $a^*$ value ($p \leq 0.05$) immediately after processing (day 0) thereafter values remained constant during the storage period of 5 days. Patras et al. (2009) reported a decrease in the $a^*$ values for both SV and WI processed carrot disks at day 0 and during storage when compared to minimally processed samples. Werlien (1998) also reported that $a^*$ value remained unchanged during storage of SV and WI processed carrots.

The values of $b^*$ (+ive = yellowness, -ive = blueness) increased after thermal processing indicating that there was a significant increase in yellowness after blanching and SV processing ($p \leq 0.05$). $b^*$ values decrease markedly during storage from day 0 to day 5 (49.7%) for SV processed samples and thereafter remained relatively constant. In the case of WI processed parsnip samples, a significant increase in $b^*$ values after WI processing when compared to minimally processed samples (57.5%, $p \leq 0.05$) was observed; thereafter values remained constant up to 3 days storage but decreased after 5 days ($p < 0.05$).

Thermal processing had a significant effect on total colour difference ($\Delta E$) of parsnip samples compared to minimally processed, both SV and WI processed
Figure 4.4. Effect of chill storage (4 °C) on levels of polyacetylenes in sous-vide processed parsnip disks in vacuumized pouches.
Figure 4.5. Effect of chill storage (4 °C) on levels of polyacetylenes in water immersion processed parsnip disks.
Figure 4.6. Effect of sous-vide processing on instrumental colour values ($L^*, a^*, b^*, \Delta E$) of parsnips disks (*Pastinaca sativa*, cv. Gladiator)
**Figure 4.7.** Effect of water immersion processing on instrumental colour values ($L^*$, $a^*$, $b^*$, $ΔE$) of parsnips disks (*Pastinaca sativa*, cv. Gladiator)
samples (p≤0.05) as illustrated in Figures 4.6 and 4.7. However, for SV processed samples, the magnitude of change in ΔE was much greater during the first five days of chill storage, than it was following either of the thermal processing steps. No significant changes in ΔE occurred in SV processed parsnip disks after 5 days chill storage. The most considerable change in ΔE values for WI samples occurred following processing which was mainly accounted for by a* and b* values changing from negative to positive i.e. samples becoming more red or less green and more yellow or less blue in colour (Figure 4.7). In contrast, no significant changes in ΔE values of WI processed samples were found to occur during chill storage (p≥0.05).

4.3.4. Degradation mechanism of polyacetylenes due to thermal treatment and its effect on the colour

Effect of thermal treatment on falcarniol type polyacetylenes could proceed via and oxidation and/or dehydrogenation route which may lead to acetylenes of the falcarniol-type as outlined in Figure 4.8. The final oxidation product outlined for falcarniol is falcarnindione (Fig 4.8). However an oxidation product on route to the formation of falcarnindione is falcarnindiol (fig 4.8) therefore this oxidation scheme could also apply to the falcarnindiol when it is present in its native form in the plant tissue. Crude extracts from parsnip for minimally and thermally processed (SVD0) were analyzed on LC-MS to investigate the presence of degradation products of falcarniol type polyacetylenes. Bohlmann et al. (1973), Hansen and Boll (1986), Christensen and Brandt (2006) have suggested several degradation pathways which may include oxidation, dehydrogenation, acetylation and loss of water. Accurate mass measurement on the LC-QTOF-MS revealed a compound with a mass of 277.1213 which fits well with the empirical formula of C_{17}H_{18}O_{2}Na (mass error <5 ppm) and this empirical formula in turn could correspond to falcarnindione a possible oxidation product of falcarniol-type polyacetylenes. This mass was not present in the extracts from minimally processed parsnip sample (Figure 4.9). In addition, the presence of a compound with a mass of 243.1754 fitted well with the empirical formula C_{17}H_{22}O (mass error ~ 2 ppm). This formula and mass fits well with dehydrofalcarniol, a dehydrogenation product of falcarniol-type polyacetylenes (Figure 4.10). Molecular ion abundance for this mass was almost two-fold higher in
the thermally processed sample when compared to the minimally processed parsnip samples. Further oxidation of dehydrofalcariinol may result in formation of ketone at carbon 3 position which is dehydrofalcariinone, a compound with an exact mass of 239.1425 which fitted well with the empirical formula C_{17}H_{18}O (mass error < 5 ppm) detected on LC-MS. Similar to the molecule mentioned earlier this mass was found both in minimal and thermally processed samples however the abundance was about two-thirds higher in the thermally processed sample (fig 4.11).
Figure 4.8. Degradation mechanisms for falcarinol type molecules
Figure 4.9. Falcarnidione in minimally processed and SV day 0 samples as identified from LC-QTOF-MS
Figure 4.10. Dehydrofalcarninol in minimally processed and SV day 0 samples as identified from LC-QTOF-MS.
Dehydrofalcarinone in minimally processed and SV day 0 samples as identified from LC-QTOF-MS

Figure 4.11.
4.4. Conclusion
In conclusion, blanching prior to sous-vide processing had the greatest influence on the retention of polyacetylenes in parsnip disks. Subsequent SV processing did not result in additional significant losses in polyacetylenes compared to blanched samples. Liquid chromatography and accurate mass mass-spectrometry revealed that degradation of falcarinol-type polyacetylenes following thermal processing may be a result of oxidation and dehydrogenation, forming oxidized forms of falcarinol-type polyacetylenes i.e. falcarindione, dehydrofalcariol, dehydrofalcarinone. Anaerobic storage of SV processed samples resulted in a decrease in falcarinol levels (p<0.05) but no change in falcarindiol levels (p>0.05). On average, WI processing resulted in a non significant but lower loss of falcarindiol as compared to SV. Falcarinol levels for WI samples were significantly higher than for SV samples. The higher losses of polyacetylenes in SV compared to WI processed samples were probably due to the combination of the inclusion of a blanching step in SV processing and the higher end point temperature. However, given the longer shelf-life afforded by SV processing in comparison to WI processing (5 days vs. 20 days), the SV technology may be the better option for retaining polyacetylenes. Falcarindiol was particularly susceptible to aerobic storage following WI processing with losses of up to 70% occurring after 5 days. Thermal processing had a significant effect on total colour difference (ΔE) of parsnip samples in both SV and WI processed disks. However, for SV processed samples the magnitude of change in ΔE was much greater during the first 5 days of chill storage than it was following either of the thermal processing steps. In general, both SV and WI processing performed equally with regard to colour retention with parsnip disks becoming darker, yellower and browner following processing and storage.

4.5. References


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Gaze, J. E., Brown, G. D. (1990). Determination of the heat resistance of a strain of non-proteolytic *Clostridium botulinum* type B and a strain of type E, heated in cod and carrot over the temperature range 70 to 90 °C. Technical Memorandum no. 592. Chipping Campden: Campden & Chorleywood Food Research Association (CCFRA), UK.


5. Effect of boiling and roasting on the polyacetylene and polyphenol content of Fennel (*Foeniculum vulgare*) bulb.

5.1. Introduction

Fennel (*Foeniculum vulgare* cv dulce) is a hardy, perennial, umbelliferous (Apiaceae) herb generally considered native to the Mediterranean areas that has become widely naturalised elsewhere. Fennel is highly aromatic with a characteristic aniseed flavour and is a staple ingredient in many dishes such as salads, soups and herbal teas (Carvalho, 2005; Tardío et al., 2005; Tardío et al., 2006; Santayana et al., 2007). The herb has many culinary and traditional medicine uses. For example, the bulbs, young shoots, leaves, flowering stems, mature inflorescences and fully ripened and dried seeds are commonly used for homemade remedies, and are said to be useful in the treatment of several complaints, specifically those of the digestive system (Camejo-Rodrigues et al., 2003; Novais et al., 2004; Carvalho, 2005; Santayana et al., 2007). Fennel has also been used in the treatment of diabetes, bronchitis and chronic coughs, kidney stones, and is considered to have diuretic, stomachic and galactogogue properties (Camejo-Rodrigues et al., 2003; Novais et al., 2004; Salgueiro, 2004; Carvalho, 2005). The possible beneficial effects of fennel consumption may be related to the presence of two groups of secondary metabolites: polyacetylenes and polyphenols. Polyacetylenes are known to occur in many members of the Apiaceae family most notably carrots and parsnips but they have also recently been reported to occur in fennel bulb (Zidorn et al., 2005). Their significance lies in their cytotoxicity against colon cancer cells, human gastric adenocarcinoma cells, leukemia (L-1210), mouse melanoma (B-16), and mouse fibroblast-derived tumor cells. (Kobæk-Larsen et al., 2005; Christensen and Brandt, 2006). The widely known polyphenols are recognized for their antioxidant properties and are known to also occur in fennel bulb (Bilia et al., 2000; Parejo et al., 2004). In common with many secondary metabolites thermal processing has been shown to affect levels of polyacetylenes and polyphenols in other foods (Patras et al., 2009; Torres et al., 2011). For example in a recent study it was found that blanching in hot water resulted in decrease in the levels of two polyacetylenes (falcarinol and falcarindiol) in parsnip disks (Chapter 5). Traditionally fennel bulb is either boiled or roasted therefore the objective
of the present study was to investigate if either of these practices affected levels of either polyacetylenes or polyphenols in the bulb.

5.2. Materials and Method

5.2.1. Chemicals
Acetonitrile (ACN), ethyl acetate, methanol and water were of HPLC grade (99.9%) and obtained from Fisher (Dublin, Ireland), Diatomaceous earth was purchased from Dionex, Surrey, UK as stated in chapters 3 and 4. The phenolic acid standards caffeic acid, gallic acid, ferulic acid and syringic acid were purchased from Sigma-Aldrich (Dublin, Ireland). Apigenin-7-O-glucoside, isovitexin and phloridzin were purchased from Extrasynthese, France.

5.2.2. Plant material and sample preparation
Fennel (Foeniculum vulgare cv dulce) was obtained from a local wholesaler (Tesco, Dublin, Ireland). Fennel bulb samples with no visible damage were selected for the experiment. The bulbs were washed and the tops, bottoms and dried outer leaves of the fennel bulbs were removed. Bulbs were cut longitudinally into 5 mm thick slices using a Berkel 800 meat slicer (Berkel company, Indiana, USA) and stored under chilled conditions (i.e. 4-8°C) until required.

5.2.3. Thermal processing of Fennel slices
Two batches of Fennel bulbs were either boiled or roasted. Boiling involved heating the samples in boiling water for 30 mins. Specifically 150g sliced fennel were boiled in 500 mL water. The boiled water was also collected to see the effect of leaching of bioactive compounds into water. Oven roasting involved cooking the fennel slices at 160°C for 15 min (until the slices were well cooked). The effect of cooking treatments was compared with the raw unprocessed (fresh) fennel. Three replicates were analyzed in each case.

5.2.4. Extraction of polyacetylenes
Extraction of polyacetylenes was performed using method described in section 3.2.4.
5.2.5. Determination of polyacetylenes using RP-HPLC
Separation and quantification of polyacetylenes was carried out by RP-HPLC using the method described in section 3.2.5.

5.2.6. Determination of polyphenols using RP-HPLC
High performance liquid chromatographic (HPLC) analysis of the filtered sample extracts in methanol was carried out according to the method of Tsao and Yang (2003). The chromatographic system (Shimadzu-Model no SPD-M10A VP, Dublin, Ireland) consisted of a pump, a vacuum degasser, a Diode-Array Detector and was controlled through EZ Start 7.3 software (Shimadzu) at 37°C. An Agilent C18 column (15 cm x 4.6 cm, 5 μm, Agilent Technologies., USA) was utilised with a binary mobile phase of 6% (v/v) acetic acid in 2 mM sodium acetate buffer (final pH 2.55, v/v, solvent A) and acetonitrile (solvent B). Solvent A was prepared first by making a 2 mM sodium acetate water solution, which was then mixed with acetic acid at a ratio of 94:6 by volume. All solvents were filtered through a 0.45 μm membrane filter prior to analysis. The flow rate was kept constant at 1.0 mL/min for total run time of 80 min. The system was run with a gradient program of 0-15% B in 45 min, 15-30% B in 15 min, 30-50% B in 5 min, 50-100% B in 5 min and 100-5% B in 10 min. The injection volume for all the samples was 10 μL. All the standards were dissolved in methanol. The detector was set at 280, 320, 360 and 520 nm for simultaneous monitoring of different groups of polyphenols. Identification of compounds was achieved by comparing their retention times and UV-Vis spectra with those of the standards in the library that was built by using the inline DAD with 3D feature. The hydroxybenzoic acid derivatives, the flavan-3-ols (including their dimers) and dihydrochalcones were quantified at 280 nm; hydroxycinnamic acid derivatives at 320 nm; flavanols at 360 nm and anthocyanins at 520 nm. In addition to polyphenols, hydroxymethylfurfural could be detected with the above mentioned method at 280 nm as described by Wijngaard and Brunton (2010).

5.2.7. Determination of total phenolics content
The total phenolics content was determined using Folin-Ciocalteu Reagent (FCR) as described by Singleton et al. (1999). The experiment was performed in two batches which included three replicates in each batch for both samples and standard. Gallic acid
at different dilutions (10-400 mg/L) was used as standard. In each replicate, 100 μL from the appropriately diluted sample extract, 100 μL methanol, 100 μL FCR and finally 700 μL Na₂CO₃ (20 %) were added together and mixed using a Vortex whirlimixer. The mixture was incubated for 20 min in the dark and room temperature. After incubation the mixture was centrifuged at 15,115×g for 3 min. The absorbance of the supernatant was measured at 735 nm using a spectrophotometer. The total phenolic content was expressed as gallic acid equivalents (GAE)/100 g dry weight (DW) of the sample.

5.2.8. Determination of radical scavenging activity (DPPH)

DPPH scavenging activity assay was performed as per the method described by Guopy et al., (1998) with a slight modification. 2,2-Diphenyl-1-picrylhydrazyl was dissolved in methanol (0.238 mg/mL). The reagent was prepared 2 hours prior to use to ensure that all the DPPH had dissolved and was stabilised. The flask containing DPPH solution was covered with aluminium foil to protect from the light and was stored in the refrigerator. For the actual assay, a 1 in 5 dilution of the DPPH stock was made using 10 mL of stock and bringing the volume up to the meniscus level with methanol in a 50 mL volumetric flask. Trolox dissolved in methanol at an appropriate dilution was used as standard. The experiment was conducted in two batches. Each batch included three replicates for both samples and standard. In each replicate 500 μL from the appropriately diluted sample extract was added to 500 μL DPPH solution. Preliminary experiments were carried out to determine the exact dilutions required. In control samples, 500 μL of methanol was added in place of sample extract with an equal volume of DPPH solution. The blank sample contained 500 μL sample extract and 500 μL methanol. The absorbance was measured at 515 nm using a spectrophotometer. The radical scavenging activity was expressed as g Trolox/100 g DW of the sample.

5.2.9. Ferric ion reducing antioxidant power (FRAP) assay

The FRAP assay was carried out as described by Stratil et al., (2006) with slight modifications. The FRAP reagent was made fresh before each experiment. It was prepared by mixing 38 mM anhydrous sodium acetate in distilled water at a pH 3.6, and adding 20 mM FeCl₃.6H₂O in distilled water and 10 mM 2,4,6-Tri-(2-pyridyl)-s-triazine
(TPTZ) in 40 mM HCl in the ratio 10:1:1. To each test assay tube, 100 μL of appropriately diluted sample extract and 900 μL of FRAP reagent were added and incubated at 37°C for 40 min in the dark. In the case of the blank, 100 μL of methanol was added to 900 μL of FRAP reagent. The absorbance of the resulting solution was measured at 593 nm in a spectrophotometer. Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic Acid) a synthetic antioxidant at concentrations from 0.1 mM-0.4 mM was used as a reference antioxidant standard. FRAP values were expressed as g Trolox/100 g DW of the sample.

5.2.10. The 2, 2’-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay
The ABTS assay was carried out according to the method of Miller et al., (1993) with slight adjustments. The principal reagents were phosphate buffered saline (80 mM/L, pH 7.4), chromogen, and hydrogen peroxide (250 μM/L). The chromogen contained metmyoglobin (6.1 μM/L) and ABTS (610 μM/L). The phosphate buffered saline was mixed with chromogen and hydrogen peroxide (H₂O₂) to give final concentrations as outlined above. For each test assay, 20 μL of the appropriately diluted sample extract was added to 1 mL of the chromogen and incubated at 37°C and the initial absorbance recorded. A 200 μL of the hydrogen peroxide was added to the mixture, the mixture re-incubated at 37°C in the dark prior to determining the final absorbance after exactly 3 min. Initial absorbance values were deducted from the corresponding final absorbance values to get the changer (or Δ) in absorbance. This value was then used to calculate antioxidant capacities in comparison with the synthetic antioxidant Trolox (0.1 mM-0.4 mM) as outlined previously in Section 5.2.9 for the FRAP assay.

5.2.11. Experimental design and statistical analysis
The effect of temperature treatment on the levels of Falcarinol (FaOH), Falcarindiol (FaDOH), Falcarindiol-3-Aacetate (FaDOAc), Caffeic Acid, Gallic acid, Apigenin-7-O-Glucoside, Ferulic Acid, Syringic Acid, Isovitexin, Phloridzin, Total Phenols, Antioxidant activity (ABTS, DPPH, FRAP) was studied. All experiments were carried out in triplicate and average values were reported. Experimental data was tested by analysis of variance and means separation was achieved using Tukey test at p<0.05
using SPSS V17. In total 24 treatments were examined: 2 for raw unprocessed samples (three replicates); 2 for boiled samples, 2 for leached water from boiled samples, 2 for roasted samples and 3 replicates for each treatment.

5.3. Results and Discussion

5.3.1. Effect of thermal processing on polyacetylene levels in fennel

Both falcarindiol and falcarninol have been reported by Zidorn et al. (2005) to occur in the fennel bulb. In the present study, we detected the presence of a peak at 42.7 min which was later confirmed to be falcarindiol-3-acetate using liquid chromatography coupled to Q-TOF mass spectrometry (data not shown; Figure 5.2). The levels of polyacetylenes in the fresh fennel were 1.16, 0.39, and 0.21 mg/100g FW for FaDOH, FaDOAc and FaOH respectively (Table 5.1). These levels are significantly lower than those demonstrated by Zidorn and colleagues (2005), who obtained concentrations of 24 mg/100g FW and 4 mg/100g FW for FaDOH and FaOH respectively. The observed differences in polyacetylene content may be attributed to the different cultivars used in the present study as the cultivar used by Zidorn and others (2005) was ‘Azoricum’ where as cultivar used in the present study was ‘Dulce’, similar results were observed for different cultivars of carrots (Chapter 4, Mercier et al. 2006). Following boiling there was a significant decrease in the levels of all the three polyacetylenes, FaDOH decreased by 82%, FaDOAc by 74% whereas FaOH by 28% respectively as compared to fresh samples. Showing that FaDOH was the most unstable of the three polyacetylenes followed by FaDOAc and FaOH; a similar trend was observed for roasting as well. Following roasting the levels of all the three polyacetylenes decreased significantly by 81%, 78% and 37% respectively for FaDOH, FaDOAc and FaOH with respect to raw unprocessed samples (fresh), this could be due to the thermal degradation of the heat sensitive polyacetylene molecules. Recent work by our group has confirmed that polyacetylenes are heat labile molecules which are easily oxidised, dehydrogenated or dehydrated into other polyacetylene type molecules (Chapter 4) upon thermal treatment (Figure 4.8).
Table 5.1. Effect of different thermal treatment on the levels of polyacetylenes in Fennel bulb.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Falcarnindiol¹</th>
<th>Falcarnindiol-3-Acetate¹</th>
<th>Falcarinol¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>1.16±0.09</td>
<td>0.39±0.02</td>
<td>0.21±0.04</td>
</tr>
<tr>
<td>Roasted</td>
<td>0.22±0.01</td>
<td>0.09±0.01</td>
<td>0.13±0.01</td>
</tr>
<tr>
<td>Boiled</td>
<td>0.21±0.01</td>
<td>0.10±0.01</td>
<td>0.15±0.01</td>
</tr>
<tr>
<td>Leached water</td>
<td>nd*</td>
<td>nd*</td>
<td>nd*</td>
</tr>
<tr>
<td>F test process²</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>LSD³</td>
<td>0.050</td>
<td>0.012</td>
<td>0.0220</td>
</tr>
</tbody>
</table>

¹ Polyacetylenes expressed in mg/100g FW
² Fischer test
³ Least significant difference
*nd- not detectable

Figure 5.1. Degradation mechanism of Caffeic acid to Ferulic acid
5.3.2. Effect of thermal processing on the level of polyphenols

The levels of phenols in the fresh fennel bulb were 206.4, 305.48, 352.04, 46.77, 229.63, 145.38 and 99.91 μg/g DW for caffeic acid, gallic acid, apigenin-7-O-glucoside, ferulic acid, syringic acid, isovitexin and phloridzin respectively (Table 5.2). With one exception, following thermal processing there was a significant (p<0.05) decrease in the levels of all polyphenols. The exception was ferulic acid, levels of which increased instead in the roasted samples (Table 5.2). A decrease in the level of polyphenols in the boiled samples was found which may be attributed to the changes in dry weight due to the combined effect of heat induced chemical oxidation and leaching out of water-soluble polyphenols. Polyphenols are thermally labile molecules; they get easily degraded upon heat treatment (Roy et al., 2007; Podsedek et al., 2008). In case of roasted samples, Maillard reactions occur at high temperatures (e.g. 100ºC), which might also contribute to the reduction of polyphenol levels. Manzocco et al. (2001) reported that polyphenolic compounds take part in Maillard reaction, a reaction that results in an increase in the Maillard reaction products and a decrease in the polyphenol level. The substantial increase of ferulic acid in roasted samples may be attributed to the degradation of caffeic acid which is a precursor to ferulic acid (Boerjan et al., 2003). A probable hypothesis is that during Maillard reaction certain phenolic acids are degraded, caffeic acid being one such phenolic monomer, may be O-methylated to ferulic acid (Rechner et al., 2001) Figure 5.1.

5.3.3. Effect of thermal processing on the levels of antioxidant activity and total phenols

The antioxidant activity of the fresh fennel extracts was 1811.73 mg Trolox/ 100g DW, 0.53 (g/L)^-1, 579.6 mg Trolox/ 100g DW, and 391.6 mg GAE/100g DW as measured by ABTS, DPPH, FRAP and TP respectively (Table 5.3). Thermal treatment resulted in a significant (p<0.05) decrease in the levels of antioxidant activity as assessed using the, DPPH and FRAP methods. An exception was noted in the case of the ABTS method which didn’t show any significant affect (or loss of activity) due to thermal treatment. Levels of individual polyphenols decreased following thermal processing and these compounds would be expected to contribute significantly to the antioxidant activity of
Boiling and Roasting of Fennel

Figure 5.2. HPLC chromatogram of Polyacetylenes for unprocessed (fresh), boiled and roasted fennel extract

- Unprocessed
- Boiled
- Roasted

1) Falcarindiol $\begin{align*} R_1 &= \text{OH} \quad R_2 &= \text{OH} \end{align*}$
2) Falcarindiol -3-acetate $\begin{align*} R_1 &= \text{CO}_2\text{H} \quad R_2 &= \text{OH} \end{align*}$
3) Falcarinol $\begin{align*} R_1 &= \text{OH} \quad R_2 &= \text{H} \end{align*}$
Table 5.2. Effect of different thermal treatment on the levels of polyphenols in Fennel bulb.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Caffeic Acid 1</th>
<th>Gallic Acid 1</th>
<th>Apigenin-7-O-Glucoside 1</th>
<th>Ferulic Acid 1</th>
<th>Syringic Acid 1</th>
<th>Isovitexin 1</th>
<th>Phloridzin 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>206±2.58</td>
<td>305.48±7.2</td>
<td>352.04±6.8</td>
<td>46.77±0.89</td>
<td>229.63±3.7</td>
<td>145.38±0.13</td>
<td>99.91±0.32</td>
</tr>
<tr>
<td>Roasted</td>
<td>163.76±2.56</td>
<td>312.51±5.13</td>
<td>94.18±1.38</td>
<td>59.23±0.03</td>
<td>139.06±1.20</td>
<td>84.6±2.02</td>
<td>18.43±0.63</td>
</tr>
<tr>
<td>Boiled</td>
<td>87.94±3.55</td>
<td>248.34±2.49</td>
<td>41.04±0.07</td>
<td>16.65±0.41</td>
<td>101.73±0.41</td>
<td>29.06±0.74</td>
<td>12.39±0.00</td>
</tr>
<tr>
<td>Leached Water</td>
<td>37.43±0.30</td>
<td>141.95±4.30</td>
<td>25.4±0.35</td>
<td>6.37±0.46</td>
<td>47.62±0.15</td>
<td>0.00</td>
<td>10.55±0.00</td>
</tr>
</tbody>
</table>

F test process 2


1Polyphenols expressed in µg/g DW
2 Fischer test
3 Least significance difference

Table 5.3. Effect of different thermal treatment on the antioxidant activity in Fennel bulb.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>ABTS 1</th>
<th>DPPH 2</th>
<th>FRAP 3</th>
<th>TP 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>1811±33.86</td>
<td>0.53±0.001</td>
<td>579.61±1.96</td>
<td>391.59±0.51</td>
</tr>
<tr>
<td>Roasted</td>
<td>1833.80±47.80</td>
<td>0.37±0.002</td>
<td>526.14±0.98</td>
<td>346.64±2.07</td>
</tr>
<tr>
<td>Boiled</td>
<td>1801±2.65</td>
<td>0.21±0.002</td>
<td>246.26±1.96</td>
<td>269.52±0.51</td>
</tr>
<tr>
<td>Leached Water</td>
<td>893.89±22.57</td>
<td>0.07±0.001</td>
<td>134.45±0.98</td>
<td>228.4±11.6</td>
</tr>
<tr>
<td>F test process 5</td>
<td>ns</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>LSD 6</td>
<td>373.08</td>
<td>0.0113</td>
<td>2.7384</td>
<td>12.51</td>
</tr>
</tbody>
</table>

1 ABTS assay for total antioxidant activity expressed as (mg TE/100g DW).
2 DPPH assay for total antioxidant activity expressed as (ARP).
3 FRAP assay for total antioxidant activity expressed as (mg TE/100g DW).
4 TP assay for total phenol expressed as (mg GAE/100g DW).
5 Fischer test
6 Least significance difference
fennel bulb extracts. Total phenol content and antioxidant capacity of the processed samples followed the same trend as for the polyphenols. The fact that levels of total phenols and the majority of individual polyphenols decreased is in line with antioxidant activity values which may indicate that phenolics were the major contributor to the total antioxidant activity of the samples tested. Other compounds may have contributed to the loss in antioxidant activity, however polyacetylenes in their pure forms have very little or no in-vitro antioxidant activity (Appendices A10).

Similar results were reported for the strong linear relationship between phenols and antioxidant activity by Shan et al., (2005) for spices. The DPPH and FRAP assays for antioxidant activity showed a similar type of response in contrast to ABTS method. The antioxidant activity of the samples could be organised in the following descending order: fresh > roasted > water immersion > leaching water (Table 5.3). Phenols are somewhat polar molecules and during boiling processes, these compounds may leach out into the water with soluble sugar. During boiling, the solubility and diffusion rate of the target compounds increases. In this study, boiling resulted in the leaching of polyphenols out from the samples into boiling water. Concerning the effect of chemical degradation of polyphenols on their antioxidant activity, the chemical oxidation of these compounds is likely to be responsible for the observed loss in antioxidant capacity. However, various studies suggest that partially oxidized polyphenols can exhibit higher antioxidant activity than that of non oxidized phenols (Manzocco et al., 2001). For this reason, further investigations are required on this topic.

5.3.4. Formation of Hydroxymethylfurfural (HMF)

Roasted samples of fennel showed the presence of a potentially harmful compound, hydroxymethylfurfural (HMF) at a concentration of 120 μg/g DW. This phenomenon can be explained from the fact that fennel bulb is rich in sugars (Escalona et al., 2003) and during roasting, formation of HMF may take place, due to Maillard reaction involving reducing sugars and amino acid as illustrated in figure 5.3. The presence of HMF was not detected in the fresh and boiled samples. Wijngaard et al. (2009) also reported that formation of HMF is accelerated at temperatures above 150°C from apple pomace during pressurized liquid extraction. This explains the reason why HMF was
Figure 5.3 Pathway of the formation of HMF from hexoses (adopted from Cämmerer et al., 1999).
found in the roasted (160°C) fennel samples in large quantity. Husoy et al. (2008) reported that important chemical modifications of carbohydrates can occur during food processing methods such as drying and heating. Many of the compounds produced give flavour and taste to the food products, but some are also suspected to be hazardous to humans.

5.4. Conclusion

The present study investigated the effect of boiling (100 °C for 30 min) and roasting (160 °C for 15 min) on the levels of these phytochemicals. Boiling resulted in a significant decrease in the levels of polyacetylenes (falcarinol, falcarindiol, falcarindiol-3-acetate) and polyphenols (caffeic acid, gallic acid, apigenin-7-o-glucoside, ferulic acid, syringic acid, isovitexin, phloridzin). The loss of polyphenols from the boiled bulbs may be in part due to leaching of these components in the water. Roasting resulted in a significant decrease in falcarindiol, falcarindiol-3-acetate, and falcarinol by 81%, 78% and 37% when compared to raw unprocessed fennel bulbs. In general levels of all polyphenols decreased in roasted samples. The exceptions were ferulic acid which showed an increase and gallic acid which did not show any decrease. In line with results for polyphenol levels, antioxidant activity decreased following thermal processing, and the presence of hydroxymethylfurfural was confirmed in roasted samples of fennel.

5.6. References


Boiling and Roasting of Fennel


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6. Effect of ultrasound and blanching pretreatments on the polyacetylene and carotenoid content of hot air and freeze dried carrot disks

6.1. Introduction

The health-promoting properties of fruit and vegetables are strongly related to their processing history (Hunter and Fletcher, 2002). For example, conditions during storage, processing and preparation have been shown to have significant effects on the content of bioactive compounds (Handelman, 2001; Kaur and Kapoor, 2001). Drying is one of the oldest methods of food preservation (Pokorny and Schmidt, 2001). Dehydrated carrots are used as an ingredient in many prepared foods such as instant soups and are an excellent ingredient for developing healthy snack foods (Lin et al., 1998). Drying can be carried out by many methods including hot air drying and freeze drying. Hot air-drying involves exposure of the product to a continuously flowing hot stream of air. Hot air drying produces dehydrated products that can have an extended shelf-life of up to a year, but the quality of conventionally dried products is usually much less than that of the original foodstuff (Ratti, 2001). This is because hot air drying leads to considerable product shrinkage, which is caused by cell collapse following moisture loss, and may also, contribute to poor rehydration characteristics of the dried product as well as unfavorable changes in colour, texture, flavor and nutrients. In contrast, vacuum freeze drying is a better method for water removal resulting in higher quality products when compared to other methods of drying (Genin and René, 1995; Irzyniec et al., 1995). Freeze drying is based on dehydration by sublimation of water from a frozen product. Due to the absence of liquid water and the low temperatures required for freeze drying, most of the deterioration and microbiological reactions are retarded resulting in a final product of high quality. Freeze drying is used in the processing of pharmaceutical products and the drying of medicinal and/or aromatic herbs, as it minimises the loss of bioactivity and flavor (Sadikoglu and Liapis, 1997; Tambunan et al., 2001). Freeze drying protects the primary structure and the shape of a product with minimal reduction of volume. Despite the obvious advantages of freeze drying, there has been poor industry uptake mainly due to the high energy costs associated with the process.
The quality of a dehydrated product depends not only on the drying conditions but also on the pretreatments employed before drying (Negi and Roy, 2001). For example, blanching involves heating a product to a high temperature for a short period and is normally carried out prior to dehydration to inactivate enzymes that may otherwise lead to formation of unacceptable colour and flavors (Mazza, 1983; Baloch and Buckle, 1997). Blanching pre-treatments are also used to improve the final texture and colour after rehydration (García-Reverter, 1994). Blanching has also been reported to reduce drying times, though thermal labile components of the product may be affected as a result of the relatively high temperatures used. In common with other thermal processes, blanching has been shown to affect the concentration of some bioactive compounds in vegetables including polyacetylenes (Hansen et al., 2003). However these studies have been far less extensive for polyacetylenes than for other bioactive compounds (e.g. antioxidants) from vegetables and have often formed part of larger studies assessing other effects such as variety, agronomic factors, etc. In addition, conflicting results have been reported by Czepa and Hofmann (2004), Kidmose et al. (2004). To date no studies on the effect of blanching followed by drying on the polyacetylene content of carrots have been reported.

Given the possible detrimental effect of blanching on the nutritional quality of some products, a need exists to develop alternative pretreatment methods that have minimal impact on the nutritional and organoleptic properties of foods (Gachovska et al., 2009). Power ultrasound is an emerging and promising alternative technology for food processing applications (Mason et al., 2005) and has been identified as a possible pretreatment to replace blanching.

While some work on ultrasound-assisted drying of vegetables such as mushrooms, brussels sprouts, cauliflower and carrots has been reported (Jambrak et al., 2007; Soria et al., 2010), no study had been carried out on the effect of ultrasound-assisted drying on phytochemicals such as polyacetylenes. The present study investigated the effect of ultrasound and blanching pretreatments on colour, polyacetylenes and carotenoid compounds in hot air and freeze dried carrot samples.
6.2. Material and methods

6.2.1. Chemicals
HPLC grade acetonitrile (ACN), ethyl acetate and water were obtained from Sigma Aldrich (UK). Diatomaceous earth was obtained from Dionex (Surrey, UK), as described in Chapter 3.

6.2.2. Sample preparation
Raw unprocessed carrots (*Daucus carota*, cv. Nerac) were obtained from a local wholesaler (Seaview, Dublin, Ireland) and stored at 4 °C for a maximum of 24 h prior to analysis. Carrots with no visible damage and of root length 15.5 ± 1.5 cm were selected for the experiment. After hand-peeling, carrots were vertically sliced into disks (5 mm thickness), using a Berkel 800 meat slicer (Berkel company, Indiana, USA), as described in Chapter 2, Section 2.2.2.

6.2.3. Ultrasound and blanching pretreatment
In order to investigate the relative influence of either ultrasound or blanching pretreatments on the quality and polyacetylene contents of carrots disks samples were either blanched or ultrasound pretreated prior to drying. Sonication was conducted for samples that were hot-air dried as well as those that were lyophilized. For ultrasound pretreatment carrot disks samples of 150 g were placed in a 400 mL beaker and 200 mL of distilled water at 25°C were added. A 1.5 kW ultrasonic processor (VC 1500, Sonics and Materials Inc., Newtown, USA) operating at 20 kHz with a 19 mm diameter probe was used for sonication. The energy input was controlled by setting the amplitude of the sonicator probe. Extrinsic parameters of amplitude (24.4, 42.7 and 61.0 μm) and processing time (3 and 10 min) were varied with pulse durations of 5 s on and 5 s off. The ultrasound probe was submerged to a depth of 25 mm in the sample as shown in Figure 8.1. All treatments were carried out in triplicate. Treatments of 3 and 10 min were selected following preliminary studies. The acoustic energy density (AED) employed in this experiment was in the range of 0.39-0.95 W/mL, as measured by calorimetry. AED was calculated at a particular amplitude level, with temperature T recorded as a function of time under adiabatic conditions using a T-type thermocouple (RS-1315, Radionics, Dublin, Ireland). From temperature versus time data, the initial temperature rise $dT/dt$ was
determined by using Eq. (1) where \((dT/dt)\) is the change in temperature over time \((\circ C s^{-1})\), \(C_p\) is the specific heat of water \((4.18 \, kJ \, kg^{-1} \, \circ C^{-1})\), and \(m\) is the mass (kg).

\[
P = mC_p(dT/dt)_{t=0}
\]  
(1)

Acoustic energy density (AED, W/ml) was calculated using Eq. (2)

\[
AED = P/V
\]
(2)

where \(P\) is power (W) and \(V\) is sample volume (mL).

For the blanching pretreatment, carrot disks samples of 150 g were placed in distilled water at 80\(^\circ\)C for 3 min.

6.2.4. Drying treatments

6.2.4.1. Hot air drying

Hot air drying of sonicated, blanched and untreated (control) samples was carried out in a laboratory scale hot air drier (Gallenkamp, Loughborough, UK) using an air velocity of 0.3 m/s to ensure that the drying rate was controlled by the internal resistance and was not dependent on mass transfer from the solid surface to the gas phase. An air temperature of 60\(^\circ\)C was selected to simulate industrial practice and minimise damage to the product. Pretreated and control samples of 150 g were placed in a perforated basket (300 \(\times\) 400 mm; size of perforation was 5 mm \(\times\) 5 mm), which was inserted in the drying chamber. Each sample was dried separately. Water loss was measured at specified time intervals (every 5 min for first 15 min, then every 15 min for the next 2 h, and every 30 min thereafter). When fully dehydrated, the samples were vacuum packed in polythene bags using a Vac Star S220 vacuum sealer (Vacquip Ltd., Dublin, Ireland) and stored at -20\(^\circ\)C.

6.2.4.2. Freeze drying

Prior to freeze drying, samples were blast frozen at a temperature of -30\(^\circ\)C for 60 min (Avon Refrigeration Co., Bristol, U.K.). Freeze drying was carried out in a A6/14 batch freeze dryer (Frozen in Time Ltd., York, U.K.) at a temperature of 0\(^\circ\)C and a pressure of 0.04 mbar for 72 h or until samples were fully dehydrated. Following this process, the samples were vacuum packed in polythene bags using a Vac Star S220 vacuum sealer (Vacquip Ltd., Dublin, Ireland) and stored at -20\(^\circ\)C.
Figure 6.1. Experimental set-up: (1) ultrasound transducer, (2) ultrasonic generator, (3) ultrasound probe (19 mm), (4) data logger, (5) temperature probe, (6) jacketed glass beaker, (7) computer and (h) depth of probe in to the water (2.5 cm).
6.2.5. Extraction of polyacetylenes
Extraction of polyacetylenes was carried out according to the method outlined in Section 3.2.4.

6.2.6. Determination of polyacetylene content using HPLC
RP-HPLC analysis of polyacetylenes was performed using an Agilent 1100 (Agilent Technologies, Wokingham, UK) series HPLC system equipped with UV-Visible detector as described in Section 3.2.5.

6.2.7. Determination of total carotenoid content using UV-Vis spectrophotometer
Total carotenoids levels were determined using the method described by Koca et al. (2007). Freeze dried carrot powder samples of 0.5 g were homogenised with 25 mL of hexane:acetone (7:3) using an Ultra-Turrax T-25 tissue homogeniser (Janke & Kunkel, IKA®-Labortechnik, Saufen, Germany) for 1 min at 24,000 rpm. The samples were thoroughly mixed with a vortex mixer (Alpha Laboratories, North York, Canada) for 20 min at 1,050 rpm and then centrifuged (MSE Mistral 3000i, Sanyo Gallenkamp, Leicestershire, UK) for 15 min at 4°C. The residue was re-extracted until it became colourless. The filtrates were combined in a separating funnel and washed with 50 mL of distilled water. The water phase was discarded and Na₂SO₄ (10 g) was added as a desiccant. The hexane phase of the extract was transferred to a 50 mL volumetric flask and hexane added to bring the contents to a final 50 mL volume. The absorbance of this solution was then determined at 450 nm using a UV-Vis spectrophotometer. External calibration with authenticated β-carotene standards solutions (0.5 μg/mL-10 μg/mL) in hexane:acetone (7:3) was used to quantify carotenoid levels in the solutions.

6.2.8. Colour measurement
The colour of samples was measured using a Hunter-Lab DP-9000 colourimeter (Hunter Associates Laboratory, Virginia, USA) as described in Section 3.2.6. Three replicates were measured and results averaged. In addition, chroma and total colour
difference (TCD) were calculated using Eqs. (1, 2), where \( L_0, a_0, b_0 \) are the values for raw unprocessed carrots.

\[
\text{Chroma} = (a^* + b^*)^{1/2} \tag{1}
\]

\[
\text{TCD} = ((L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2)^{1/2} \tag{2}
\]

6.2.9. Experimental design and statistical analysis

The effects of two drying pretreatments namely blanching and ultrasound on the levels of falcarinol (FaOH), falcarindiol (FaDOH), falcarindiol-3-acetate (FaDOAc), total carotenoids, \( L^*, a^*, b^* \), chroma, and total colour difference (TCD) were studied. In total 72 samples were analyzed in three batches, of which 54 samples were sonicated (3 amplitude \( \times 3 \) replications \( \times 3 \) batches \( \times 2 \) drying treatments) and 18 were blanched (1 blanched \( \times 3 \) replications \( \times 3 \) batches \( \times 2 \) drying treatments). Experimental data obtained were statistically analyzed using statistical software (SAS V.9.1, SAS Institute, NC, USA). Means were separated by least significant difference (LSD) using Tukey’s test. Significance was accepted at \( p<0.05 \).

6.3. Results and Discussion

6.3.1. Effect of ultrasound and blanching pretreatments on the polyacetylene content of hot air and freeze dried carrot disks

The levels of the 3 major polyacetylenes i.e. falcarinol (FaOH), falcarindiol (FaDOH), and falcarindiol-3-acetate (FaDOAc) in the fresh carrots (peeled) were found to be 112.06, 64.53 and 11.85 \( \mu g/g \) DW respectively. The values obtained are similar to those reported by Kidmose et al. (2004), but significantly lower than values of 359, 154 and 77 \( \mu g/g \) DW for FaOH, FaDOH and FaDOAc respectively previously reported in chapter 3. The observed differences in polyacetylene content reported are mainly attributed to genotypic variation (Mercier et al., 2006; Rawson et al., 2010a). The degree of retention was calculated with respect to fresh carrots (peeled). The degree of retention of polyacetylenes in carrots followed by ultrasound pretreatment followed by hot air drying (UPHD), ultrasound pretreatment followed by freeze drying (UPFD) and blanching pretreatment followed by hot air drying (BHD) are presented in Figures 8.2 and 8.3. Degree of retention is defined as the % of compounds retained with respect to freeze dried samples that had not been blanched.
or received any pretreatment. After BHD, the levels of retention of, FaDOH, FaDOAc and FaOH were 36.4%, 77.9% and 25.4% respectively compared to control. (Figure 8.2, p< 0.05). This is most likely due to the relatively high temperatures required for blanching treatment (80°C, 3 min) which could lead to oxidative and thermal degradation (Rawson et al., 2010b). Kidmose et al. (2004) reported similar behavior for polyacetylenes following blanching at 90°C and short holding times. On the other hand UPHD samples sonicated at the highest treatment conditions (61 μm and 10 min) had higher retention levels for all 3 polyacetylenes compared to BHD and hot air dried samples only (Figure 8.3, p<0.05). In the case of FaOH, the most biologically active of the 3 polyacetylenes, pretreatment with ultrasound at amplitudes of 42.7 and 61.0 μm resulted in significantly higher levels of retention compared to BHD samples; 60 and 64.4% higher at 3 min treatment time, whereas it was 37.2 and 86.2% higher at 10 min treatment time. In general, higher ultrasound amplitudes and longer holding times resulted in improved retention of polyacetylenes compared to hot air dried samples with no pretreatment. This increase in the retention of polyacetylenes may arise from an increase in the extractability of the compounds, while the different effects observed for different holding times could be a reflection of the activation energy required to dissociate polyacetylenes from pectin-rich cell walls. Improved extraction efficiency following sonication has been attributed to the propagation of ultrasound pressure waves, with induced cavitation and high shear forces resulting in increased mass transfer (Jian-Bing et al., 2006; Vilkhu et al., 2008). Simal et al. (1998) suggested that the degassing effect observed under sonication may be similar to that observed under vacuum treatment, which can enhance diffusion into pores on the surface and may explain the enhanced extractability. In addition the greater retention of polyacetylenes at higher amplitudes and holding times could be related to improved denaturation of enzymes responsible for their degradation.

In general, freeze drying regardless of pre-treatment employed resulted in higher retention of all 3 polyacetylenes than hot air drying. This was particularly evident for FaOH where levels of retention in UPFD samples with a holding time of 10 min were 44.3, 37.8 and 28.1% higher than BHD samples at amplitudes of 24.4, 42.7 and 61.0 μm respectively. Similar trends were observed for retention of FaDOH and FaDOAC across all ultrasound pretreatments (Figure 8.3). In many cases, the
**Effect of Drying and Pretreatments**

![Graph showing carotenoids and their retention after drying and pretreatments](image)

Figure 6.2a: Retention of carotenoids, FaDOH, FaDOAc and FaOH of carrot disks pre-treated with ultrasound for 3 min followed by hot air drying.

Where,

- BHD: blanched hot air dried
- UPHD: ultrasound pre-treated hot air dried

Control (freeze dried) | Hot Air dried only | BHD
---|---|---
UPHD-24.4μm | UPHD-42.7μm | UPHD-61.0μm
Effect of Drying and Pretreatments

Figure 6.2b: Retention of carotenoids, FaDOH, FaDOAc and FaOH of carrot disks pre-treated with ultrasound for 3 min followed by freeze drying

Where,

BHD: blanched hot air dried, UPFD: ultrasound pre-treated freeze dried
Effect of Drying and Pretreatments

Figure 6.3a: Retention of carotenoids, FaDOH, FaDOAc and FaOH of carrot disks pretreated with ultrasound for 10 min followed by hot air drying.

Where,

BHD: blanched hot air dried, UPHD: ultrasound pre-treated hot air dried
Figure 6.3b: Retention of carotenoids, FaDOH, FaDOAc and FaOH of carrot disks pretreated with ultrasound for 10 min followed by freeze drying

Where,
BHD: blanched hot air dried, UPFD: ultrasound pre-treated freeze dried
levels of retention for freeze dried samples with ultrasound pretreatment was higher than for freeze dried samples with no pretreatment (designated as 100% retention). This finding could be due to increased extractability induced by cavitations in ultrasound treated samples as discussed above.

6.3.2. Effect of ultrasound and blanching pretreatments on the carotenoid content and colour of hot air and freeze dried carrot disks

Carotenoids are coloured pigments which are responsible for imparting the characteristic orange colour to carrots. In the present study levels of total carotenoids in the fresh carrots were 789.55 µg/g DW; these levels are higher than those reported by Patras et al. (2009). This result may be due to variations in cultivar selection. Unlike the case for polyacetylenes there was no significant difference (p>0.05) in carotenoid levels between BHD and hot air dried samples without pretreatment. There was however, a significantly (p<0.05) higher retention of carotenoids in UPHD (24.4 µm amplitude level, treatment time 10 min) than BHD samples. In addition the total carotenoids loss for hot-air dried sample compared to freeze dried samples (control) was 28.4%.

Carotenoids are sensitive to heat, oxygen, light, and enzymes. For example Mudahar et al. (1989) reported that losses in total carotene content during carrot drying processes were between 30 and 60%. Park (1987) reported carotene losses of 54% and 62% for vacuum and microwave dried carrots, respectively. Carotenoid content is an important parameter in determining the final quality of thermally-dehydrated vegetables such as carrots, as it is a determining factor in colour and nutritional quality.

The lowest ultrasound amplitude level of 24.4 µm resulted in the highest retention of carotenoids in UPFD carrots for both 3 and 10 min treatment times. No significant differences were observed between freeze dried (without any pretreatment) and UPFD samples after a 3 min treatment time demonstrating that insignificant losses occur during freeze drying (Figures 6.2 and 6.3).

The $L^*$ values of UPFD samples were significantly higher compared to fresh, BHD and UPHD samples. Similarly, Lin et al. (1998) observed higher $L^*$ value for freeze dried carrot slices compared to vacuum microwave and air dried carrot slices.
Figure 6.4: Effect of ultrasound and blanching pretreatments on color parameters of a) $L^*$, (b) $a^*$, (c) chroma and (d) TCD of dried carrots disks for 10 min.
A significant difference was noted in perceivable colour (TCD) as observed for BHD, UPHD and UPFD compared to fresh samples with no significant difference between BHD and UPHD. However, UPFD samples showed significantly \((p<0.05)\) higher TCD compared to UPHD and BHD samples as shown in Figure 6.4. Colour parameters \((L^*, r=0.73; p<0.0001); a^* (r=0.54; p<0.001); \) chroma \((r=0.45; p<0.01)\) and TCD\((r=0.66; p<0.0001)\) strongly correlated with carotenoids contents.

Colour parameters were most affected by hot air drying as colour degradation of carotenoids depends more on temperature and oxygen (Cui et al., 2004). Carotenoid-rich vegetables, such as carrots, when exposed to air and high-temperature, exhibit a distinct colour degradation resulting from the high degree of unsaturation in their structure, which makes them susceptible to oxidative reactions as well as to isomerization of trans-carotenoids to the less highly coloured cis-forms. Lipoxygenases are the major enzymes involved in carotenoid degradation. They are thermostable and capable of forming reactive radicals that destroy carotenoids (Cui et al., 2004).

6.4. Conclusions

From the present study, it may be concluded that freeze drying of carrot results in higher retention of polyacetylenes, carotenoids and colour parameters than hot air drying. It can also be concluded that power ultrasound as a pretreatment has the potential to enhance the retention of bioactive compounds by providing the activation energy required to dissociate bound polyacetylenes from the pectic-rich cell wall of carrots. Further research is required to optimize the retention of bioactives by varying ultrasonic processing parameters such as power level, treatment time and temperature.

6.5. References


Effect of Drying and Pretreatments


Effect of Drying and Pretreatments


7. High pressure-temperature degradation kinetics of polyacetylenes in Carrots

7.1. Introduction
Carrots are often consumed raw but more commonly are thermally processed and in this form can be added to a wide variety of products, including canned, frozen, dehydrated soups, baby foods and mixed vegetables. While thermal processing renders carrots more stable and digestable it has been shown to affect the concentration of some bioactive compounds in vegetables including polyacetylenes (Hansen et al., 2003; chapter 4, 5, 6). However for polyacetylenes, these studies have been far less extensive than for the studies related to other bioactive compounds from vegetables, and have often formed a part of larger studies assessing other effects such as variety and agronomic factors. In cases where thermal processing has been shown to affect levels of potentially beneficial compounds, the use of alternative methods is often examined. Of these high hydrostatic pressure has shown the most potential for maximizing retention of bio-active compounds (Patras et al., 2009). High hydrostatic pressure processing uses water as a medium to transmit pressures from 200 to 800 MPa to foods resulting in a reduction in microbial loads and thus extending shelf-life. It is also possible to heat the pressure-transmitting water to speed up processing times or increase the stability of the product (Patterson et al., 1996; Meyer et al., 2000). To date no study has investigated the effect of high pressure on polyacetylenes in food. Therefore, an objective of the study presented in this chapter was to assess the effect of high pressure-temperature processing on polyacylene retention as compared to thermal processing. In addition, no information is available on the kinetics of polyacetylene degradation in response to processing. Hence, in the present study the effect of high pressure-temperature on polyacetylene degradation in carrot (*Daucus carota* L.) has been investigated on a kinetic basis.
7.2. Materials and Methods

7.2.1. Chemicals
Acetonitrile (ACN), ethyl acetate and water were of HPLC grade (99.9%) and obtained from Lennox (Dublin, Ireland).

7.2.3. Plant material and sample preparation
Fresh carrots (*Daucus carota*, L.) were obtained from a local wholesaler (Donelly’s, Dublin) and stored at 4°C for a minimum of 24h prior to analysis. Carrots with no visible damage and of root size 15.5±1.5cm were selected for the experiment. After hand peeling, carrots were sliced in to disks (5mm) using a Berkel 800 meat slicer (Berkel company, Indiana, USA) as described earlier in Chapter 2, Section 2.2.

7.2.4. High Pressure Processing of Carrots
Two lots of (150g each) carrot slices were placed in plastic bags (polyethylene [PE] bags, 15 × 20 cm) and vacuum packed using a Vac Star 220 vacuum sealer (Vicquip Ltd., Dublin, Ireland). Samples were placed in a high pressure vessel (100 mm internal diameter × 254 mm internal height, Pressure Engineered System, Belgium) filled with a mixture of water and rust inhibitor (Dowcal N, 60% v/v in distilled water). Conditions for high pressure-temperature treatment of the disks were chosen according to the recommendations of Yuste et al. (2001). Accordingly carrot slices were subjected to pressures of 400, and 500 MPa for 0, 2, 5, 10, 20, and 30 min at temperatures of 50, 60 and 70°C. The time taken to reach the target pressure was approximately (60-100 s) and depressurization took 10 s. Immediately after processing the samples were cooled to 4°C. Samples were then blast frozen at -24°C (Avon Refrigeration Co., Bristol, U.K.), freeze-dried (model A6/14, Frozen in Time Ltd., York, U.K.), vacuum packed using a Vac Star S220 vacuum sealer and stored in polythene bags at -20°C prior to chromatographic analysis.

7.2.5. *Sous-vide* (SV) Processing of Carrots
*Sous-vide* processing of carrots were performed by the method as described in section 4.2.3 for parsnips. However in this study no storage was conducted following SV processing.
7.2. 5 Extraction of polyacetylenes
Extraction of polyacetylenes was performed using method described in section 3.2.4.

7.2. 6. Separation and quantification of polyacetylene
Separation and quantification of polyacetylenes was carried out by RP-HPLC using the method described in section 3.2.5.

7.2.7. Determination of kinetics parameters
The behavior of polyacetylenes following processing was modeled using the Weibull model (Eq. 1). Since the 0 order and 1st order degradation kinetic gave a lower $R^2$ value (50-65%) compared to Weibull distribution model which gave high $R^2$ values (>90%)

$$C_t = C_0 e^{-(kt)^\beta}$$  \hspace{1cm} (1)

Where,
$C_t$ is the polyacetylene concentration at a time $t$, $C_0$ is the initial polyacetylene concentration; $k$ is the degradation rate constant (min$^{-1}$) and $\beta$ (dimensionless) is the shape constant determining the shape of degradation curve.

The Weibull model degradation rate constant $k$ gives the rate at which the degradation of the compound takes place, the higher the $k$ value the lower the stability of compound. If the rate of degradation increases over time then the shape factor ($\beta$) is greater than 1 (i.e. formation of a shoulder), if the rate decreases over treatment time then the shape factor of the curve ($\beta$) is less than 1 (i.e. formation of a tail) and if rate is constant over time the shape factor ($\beta$) is 1 (i.e. first order degradation; Cullen et al., 2009).

7.2.8. Statistical Analysis and experimental design
Data were analyzed by Minitab v15 using an ANOVA GLM procedure. The effects of temperature (50, 60, 70 °C), Pressure (400, 500 MPa) and Treatment Time (0, 2), on polyacetylene content in carrot disks were studied. In total 30 treatments were
examined: three temperatures (50, 60, 70 °C) × two pressures (400, 500 MPa) × 5 treatment Times (0, 5, 10, 20, 30 min) × 3 replicates per treatment were analyzed.

7.3. Results and discussion

7.3.1. Effect of high pressure – temperature treatment

Levels of the 3 major polyacetylenes falcarinol (FaOH), falcarindiol (FaDOH), and falcarindiol-3-acetate (FaDOAc) in the fresh carrots disks were 253.6, 48.18, 18.81 µg/g DW respectively. The levels reported in this work were lower than those reported in section 3.3.1, 6.3.1, 7.3.1 but within the range of those reported by Kreutzmann et al. (2008). In comparison to fresh carrots all high pressure-temperature treated samples resulted in a significant decrease (p<0.05) in the levels of the 3 major polyacetylenes in carrot (Figure 7.1, 7.2, 7.3). In fact all the three parameters ie. pressure, temperature and time were found to have significant effect (p<0.05) on the levels of polyacetylenes (FaOH, FaDOH, FaDOAc).

High pressure treatment can be used to improve microbiological quality thus extending shelf-lives during refrigeration and, in some cases, at room temperature storage (Yuste et. al. 2001). Because of the pressure resistance of spores and some food deteriorating enzymes at room temperature, a combination of high pressure with other treatments (e.g., heat treatment) is sometimes needed to obtain sterile and shelf-stable food products (Van Loey et al. 1998). Hence, the present study investigated the pressure stability of polyacetylenes at elevated temperatures (50, 60, 70°C). Weibull modelling was used to quantify the rate of degradation of the three major polyacetylenes following high pressure-temperature processing treatment (Eq. 1). The Weibull model is flexible owing to the inclusion of a shape constant, in addition to the rate constant and has been employed to describe microbial, enzymatic and chemical degradation kinetics (Cunha et al., 1998; Manso et al., 2001; Tiwari et al., 2009). Rate constants for the polyacetylene degradation process at all temperatures and pressures are presented in Table 7.1. The model fitting of high pressure-temperature degradation of falcarinol (FaOH), falcarindiol (FaDOH), and falcarindiol-3-acetate (FaDOAc) is depicted in Figures 7.1, 7.2, 7.3. The model had a high $R^2$ value (> 0.85) and low RMSE values (<0.05) indicating a good fit of the experimental data with the model.
As pressure increased the degradation rate of polyacetylenes increased (Table 7.1). For example at a temperature of 50°C the k value at 400 and 500 MPa was $0.89 \times 10^{-2}$ min$^{-1}$ ($R^2 = 0.98$) and $1.1 \times 10^{-2}$ min$^{-1}$ ($R^2 = 0.96$) respectively for FaOH, indicating that an increase in pressure at elevated temperature resulted in an increased degradation rate for FaOH. A similar trend was noted for the other two polyacetylenes at this temperature. The rate of degradation for FaDOAc was highest of the three polyacetylenes investigated when high temperature was held constant and pressure increased, indicating that this compound may be barosensitive at high temperatures. In relation to overall pressure sensitivity, the rate of degradation of FaOH was greater than FaDOH but not as great as FaDOAc with some exceptions; at 60°C treatment temperature, the k value at 400 and 500 MPa was $0.98 \times 10^{-2}$ min$^{-1}$ ($R^2 = 0.92$) and $1.06 \times 10^{-2}$ min$^{-1}$ ($R^2 = 0.80$) for FaOH, $1.23 \times 10^{-2}$ min$^{-1}$ ($R^2 = 0.97$) and $1.69 \times 10^{-2}$ min$^{-1}$ ($R^2 = 0.98$) for FaDOH and $1.44 \times 10^{-2}$ min$^{-1}$ ($R^2 = 0.99$) $1.87 \times 10^{-2}$ min$^{-1}$ ($R^2 = 0.96$) for FaDOAc respectively (Table 7.1). The data indicate that FaDOH was degraded fastest followed by FaDOAc and FaOH. These observations suggest that, in general, the application of high pressure-temperature may result in lower retentions of polyacetylenes, depending on the severity of the treatment conditions. The highest loss of FaOH (~44%), the most bioactive compounds of the three polyacetylenes studied was found to occur at 500MPa, 70°C for 30 min. No previous studies have investigated the pressure-temperature stability of polyacetylenes in a foodstuff, nevertheless many other lipophilic bioactive compounds such as carotenoids, vitamins and chlorophyll have been studied. Tauscher (1998) and De Ancos et al. (2000) and reported that high pressure treatment (600 MPa) at elevated temperature (75°C) for 40 min led to only a 5% loss of carotene in carrot based products. In another study Vitamin A acetate degradation in ethanol solution was found to be more pronounced with increasing pressure and temperature (Tauscher 1999). Van Loey et al. (1998) studied the effect of high pressure-temperature on chlorophyll content in broccoli juice; these authors found a significant decrease in the chlorophyll content at temperatures higher than 50°C. At constant pressure (400/500 MPa) the rate of degradation increased as the temperatures increased, especially in the interval from 60°C to 70°C. The temperature instability of polyacetylenes has also been noted in other
studies (Hansen et al. 2003, chapter 3, 4). In Chapter 3 it was reported that FaDOH (~52% loss) was the most susceptible to thermal

**Table 7.1** Rate constants \((k)\) for falcarinol (FaOH), falcarindiol (FaDOH) and falcarindiol-3-acetate (FaDOAc) following a range of pressure-temperature combinations.

<table>
<thead>
<tr>
<th>Pressure</th>
<th>Temperature</th>
<th>FaOH (k \left(10^{-2} \text{min}^{-1}\right))</th>
<th>Shape ((\beta))</th>
<th>RMSE</th>
<th>(R^2)</th>
<th>(R^2_{adj})</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>70</td>
<td>2.25 ± 0.01</td>
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Figure 7.1 Relative changes in Falcarniol content following high pressure treatment at
a) 400 MPa   b) 500 MPa and temperatures (•) 50 °C, (▲) 60 °C, (■) 70 °C.
Figure 7.2 Relative changes in Falcarindiol content following high pressure treatment at a) 400 MPa  
and temperatures (●) 50 °C, (▲) 60 °C, (■) 70 °C. 

### Figure 7.2
Relative changes in Falcarindiol content following high pressure treatment at a) 400 MPa and temperatures (●) 50 °C, (▲) 60 °C, (■) 70 °C.
Figure 7.3 Relative changes in Falcarindiol-3-acetate content following high pressure treatment at a) 400 MPa  b) 500 MPa and temperatures (●) 50 °C, (△) 60 °C, (■) 70 °C.
degradation followed by FaOH (~44% loss), and FaDOAc (~44% loss) at atmospheric pressure. Van Loey et al. (1998) reported a similar observation for chlorophyll in broccoli juice and this was in accordance to Arrhenius law that degradation rate increased with temperature at all pressures tested.

The Weibull model shape factor ($\beta$) which represents the shape of the curve may be used to describe the degradation pattern of falcarinol-type polyacetylenes with respect to treatment time as has been suggested by Manso et al. (2001) for ascorbic acid. In general $\beta$ was found to be less than 1 indicating that the degradation rate decreased over treatment time. Of all the three polyacetylenes molecules FaDOH had the highest value of $\beta$ at 70°C and 400-500 MPa indicating that the decrease in the rate of degradation of FaDOH over time was lower than FaOH and FaDOAc. This study suggests that FaDOH is the most unstable polyacetylene compound to a high pressure-temperature treatment processing. The highest pressure-temperature combination which gave maximum retention at time 10, 20 or 30 min, for FaOH was 400 MPa at 50°C and 60°C for 10 min; for FaDOH these conditions were 400 MPa, at 50°C for 10 min while for FaDOAc the treatment parameters were 400 MPa, at 50°C for 10 min, respectively. From the point of view of treatment time, a longer treatment time led to a higher decrease of polyacetylenes at all high pressure-temperature combinations. After 2 min treatment time the retention of FaOH was 97-90%, FaDOH 94-91% and FaDOAc 93-86% for all pressure-temperature combinations.

7.3.2. **Comparison with SV processed carrot samples**

The levels of polyacetylenes in SV processed carrot disks samples were analysed and compared with high pressure-temperature processed samples (Figure 7.4). Following SV processing a significant decrease ($p<0.05$) was observed in the levels of all the three polyacetylenes. This is in line with previous results on parsnips (see Chapter 4; Rawson et al., 2010b). In addition Hansen et al. (2003) reported that there was 70% decrease in the levels of falcarinol following thermal processing such as boiling of carrot pieces in water for 12 min, and a similar reduction was observed following steam blanching of carrot cubes. The probable cause of the decrease in the levels of polyacetylenes
following thermal processing can be attributed to oxidation, dehydrogenation, and water loss (see Chapter 4; Rawson et al., 2010b). FaDOH was found to be retained the least following sous-vide processing again confirming the fact that this compound is more susceptible to thermal treatment than FaOH and FaDOAc.

In terms of % retention, polyacetylenes were retained less in SV processed samples compared to high pressure-temperature processed samples, especially for samples treated at lower temperatures of 50 or 60°C. At a temperature of 70°C, the combined effect of temperatures and pressure resulted in lower levels of polyacetylenes than at other temperatures, but levels were still higher than SV processed samples (Fig 7.4). The reason why polyacetylenes are better retained using a combination of pressure and temperature is probably governed by two factors. Firstly high pressure treatment has been shown to have limited effect on covalent bonds as would be contained in all three polyacetylenes under investigation (Balny et al., 1997); Secondly high pressure treatment has been shown to increase the extractability of many compounds (De Ancos et al., 2002; Oey et al., 2008). While it is clear that some polyacetylenes are barosensitive this is probably offset by increases in extractability result in an overall better retention for high pressure- temperature treated samples.
Figure 7.4 Percentage retention in the levels of polyacetylenes in control and processed carrot samples

Where,  
SV: sous-vide processed  
HPT: high pressure-temperature processed
7.4. Conclusion

The retention of polyacetylenes in high pressure-temperature processed samples was highly dependent on treatment time, operating temperature and pressure. Of the three polyacetylenes FaDOAc was found to be most barosensitive and FaDOH most thermolabile. When compared to SV processed samples, high pressure-temperature processed samples in general showed higher retention of polyacetylenes. Scope exists to optimize these parameters (high pressure, temperature, treatment time) to potentially increase the retention of polyacetylenes. All these optimisations could come in form of recommendations for processors and should take into account the microbiological safety, the organoleptic quality including texture, as well as the equal retention of other micronutrients present in carrots such as vitamins, flavonoids and minerals.

7.5. References


Gaze, J. E., and Brown, G. D. (1990). Determination of the heat resistance of a strain of non-proteolytic Clostridium botulinum type B and a strain of type E, heated in cod and carrot over the temperature range 70 to 90 °C; Technical Memorandum no. 592; Chipping Campden: Campden & Chorleywood Food Research Association (CCFRA): U.K.


8. Influence of unit operations on the levels of polyacetylenes in minimally processed carrots and parsnips: an Industrial Trial

8.1. Introduction

In recent times considerable effort has been placed by food processors on the development of innovative Ready-to-Eat food (RTE) products with fresh-like characteristics that match modern urban lifestyles where time for food preparation is limited. As a result, nowadays consumers can find a variety of products ranging from ready-to-heat cooked whole meals to freshly prepared chilled salad snacks. Many consumers prefer uncooked, minimally processed vegetables as they perceive these to be the healthiest option and are cognisant of the benefits of vegetables-rich diets containing micronutrients such as vitamins, flavonoids (Lui, 2003; Trichopoulou et al., 2003). Carrots are often a key ingredient in RTE vegetable products representing up to 20% of the total weight and they can be present many forms such as shredded, sliced in disks or cut in cubes and batons. Carrots are well known sources of many key nutrients such as vitamin C, β-carotene and flavonoids, and many studies have investigated their retention during preparation or after processing has been undertaken (Basu et al., 2001; González-Aguilar et al., 2009). As outlined elsewhere carrots, contain in minor quantities a group of bioactive aliphatic C17-polyacetylenes (Bohlmann et al., 1973; Hansen and Boll, 1986; Czepa and Hoffmann, 2003; Rai et al., 2011). Since the possible biological relevance of polyacetylenes has only recently come to light less is known about their stability during processing than for other more established phytochemicals such as polyphenols and carotenoids.

The majority of the studies that have examined the effects of processing on polyacetylenes have concentrated on full scale processing methods such as boiling and sous-vide processing (Hansen et al., 2003; Kidmose et al., 2004) including those outlined in Chapters 3 and 4 of the present work. In addition, studies evaluating the polyacetylene content in various parts of carrot root (to study anatomical distribution) indicate that the “epidermis” (root peel) is rich in specific polyacetylenes (Czepa and Hoffmann, 2003; Kidmose et al., 2004; Baranska and Schulz, 2005) although the results seem to be variety dependent (Mercier et al., 1993). This indicates that mechanical processes, or so called “unit operations”, in
raw vegetable processing, including “peeling”, “cutting or “slicing” and “washing” may influence the concentration of polyacetylenes in the final product. Despite this, few studies have addressed the effect of minimal processing on polyacetylene content and those that have addressed it (Zidorn et al., 2005) have been carried out on samples produced in a laboratory setting, using equipment not representative of that used in an industrial setting. The present study aims to address this gap in knowledge in conjunction with an industrial partner (WonderFoods Ltd.) by examining polyacetylene levels in carrot and parsnips subjected to three consecutive unit operations (peeling, cutting, and washing) where samples were taken in every point of their processing line. In addition, the stability of these compounds during storage at chill temperatures under typical retail conditions was assessed. The ultimate objective is to provide processors with recommendations for the maximum retention of polyacetylenes in minimally processed carrots and parsnips following processing and chill storage.

8.2. Materials and Methods

8.2.1. Chemicals

Acetonitrile (ACN) and water were of HPLC grade and obtained from Fisher Scientific (Dublin, Ireland). Diatomaceous earth was obtained from Dionex (Surrey, UK).

8.2.2. Preparation of vegetables

Fresh carrots (Daucus carota, cv. Nantes) and parsnips (Pastinaca sativa cv. Gladiator) were purchased from a local wholesaler (SeaView Vegetables Co., Dublin, Ireland). The vegetables were carefully inspected selecting only those that were of uniform size and free from defects. The average weight of the carrot roots selected was 63.5 ± 18.1 g (mean ± s.d.), and the average length was 15.5 ± 2.8 cm. The average weight of parsnip roots selected was 192.9 ± 38.2 g, with an average length of 18.1 ± 2.9 cm.

8.2.3. Minimal processing of carrots and parsnips

Figure 6.1 illustrates the protocol used to produce the carrot and parsnip products. Carrot and parsnips selected for processing were transferred to the processing hall of WonderFoods Ltd. (Roslin Food Park, St. Margaret’s, Co. Dublin,
Ireland) where minimal processing was conducted in early March 2009. Good Manufacturing Practices (GMP) was followed (disinfection, working temperature of 7°C). All processing was performed on Eillert Food Processing Equipment (Machinefabriek Eillert BV, Winterswijk, The Netherlands). Abrasive peeling was performed using an Eillert Knife peeling machine (type B25RVS) which was fitted with rotating carborundum drums. Time in contact with the drums and thus depth of peeling was constant at all times. Cutting or slicing was achieved in an Eillert stripping/dicing machine (model BL-1000 A) to produce disks, batons, shreds and cubes. Washing was performed in an Eillert series GWB Vegetable washing machine (series GWB). The vegetables were manually fed into the standard 50 L tank and dipped in 5% (v/v) aqueous solution of chlorine for 5 min with stirring. Four types of carrot products were prepared: a) carrot disks of 0.5 cm thickness, b) batons (5.0 cm × 1.0 cm w × 1.0 cm b), c) cubes (1.0 cm × 1.0 cm × 1.0 cm) and shreds (approx. 3.0 cm × 0.2 cm w × 0.2 cm b). For the production of carrot disks and batons the sequence of operations was: peeling, cutting and washing. For the production of carrot cubes and shreds, washing was carried out prior to cutting (Fig. 8.1). For parsnips a portion of 20 kg of selected parsnips was used to prepare 3 products i.e., disks, batons and cubes of identical sizes to the carrots (Fig. 8.1). Parsnip shreds were technically not possible to produce due to their softer texture compared with carrot. Peeling of parsnip was performed by hand as they were not compatible with the abrasive peeling machine.

Carrot and parsnip minimally processed products were placed (between 95-100 g) were placed in individual polyethylene (PE) plastic bags (15 × 20 cm) and stored in a chilled display cabinet with 24 h fluorescent light maintained at 2-4°C and a relative humidity of 95%. The conditions were similar to the typical conditions in retail stores. Finally they were freeze dried at every sampling point during processing and storage for quantification on RP-HPLC.

8.2.4. Extraction of polyacetylenes

Extraction of polyacetylenes was performed using the method described in Section 3.2.4.
8.2.5. Determination of polyacetylene content using HPLC

Separation and quantification of polyacetylenes was carried out by RP-HPLC using the method described in Section 3.2.5.

8.2.6. Statistical Analysis

Experimental data was obtained in triplicate, differences were considered significant at p<0.05. Analysis of variance (ANOVA) was performed using IBM SPSS Statistics version 19 (IBM Corp., Sommers, New York) to show the significance in carrots and parsnips during processing and storage. In total 204 samples were analysed, of which 114 were carrot and 90 were parsnips. For carrots: 3 fresh unprocessed; 3 peeled carrot; 3 × 4 minimally processed (baton, disks, cube, shreds); 3×4 washed minimally processed; 3 × 7 × 4 batches from storage were analysed. The same sample lots were prepared for parsnips, the only difference was that no shredded samples were prepared for the parsnips. All of the treatments were done in triplicate.

8.3. Results and Discussion

8.3.1. Occurrence of polyacetylenes in both carrot and parsnip extracts

The polyacetylenes falcarniol (FaOH), falcarindiol (FaDOH) and falcarinoldiol-3-acetate (FaDOAc) were identified in carrot extracts by comparison of their retention times with those of MS and NMR authenticated standards which had been isolated and purified from freeze dried carrot powder, as described by in Chapter 2. Parsnips were found to contain only two polyacetylenes (FaDOH, FaOH); this is in accordance with Chapter 4 of this research (see also Rawson et al., 2010b) and a previous study (Zidorn et al., 2005), although no obvious physiological reason is known (Degen et al., 1999; Christensen, 2011). Parsnips contained a higher amount of FaDOH and FaOH compared to carrots as is evident from Figures 8.2 and 8.3. Levels of the individual polyacetylenes in the fresh unprocessed carrots were 158.3, 55.5, 277.5 µg/g DW for FaDOH, FaDOAc, FaOH, respectively, and in case of parsnips concentrations of 252.1, 330.7 µg/g DW were determined for FaDOH, FaOH respectively. These values are in the range of those reported by Zidorn et al. (2005), Christensen and Kreutzmann (2007), section 7.3.1 and section 9.3.1 but values as high as 359 µg/g DW has also been reported for FaOH (section 3.3.1) in carrots, while values for parsnips were much lower than those reported by Zidorn et
al. (2005) and those reported in section 4.3.1. Levels of polyacetylenes in Apiaceae root vegetables have been shown to vary due to several factors including cultivar type (Mercier et al., 1993), root size (Kidmose et al., 2004) and environmental and agronomic conditions (Søltoft et al., 2010).

**Figure 8.1.** Minimal processing of carrot and parsnip – experimental design of the industrial trial.
8.3.2. The effect of unit operations in minimally processed carrots

Figure 8.2 presents level of total and individual polyacetylenes in carrots as sampled sequentially at different sampling points (SPs) along the processing line as illustrated in Figure 8.1. For carrots, the total polyacetylene levels of processed carrots were significantly lower (p<0.05) at the end of the minimal processing (Figure 8.2) in comparison to unprocessed samples. This decrease in polyacetylenes was more apparent when carrots were cut in disks and less so when they were prepared in cubes, or was shredded, although these differences were not significant (Fig. 8.2). Of all the unit operations applied on carrots, “peeling” had the most significant impact on the polyacetylene content. In terms of individual polyacetylenes, the level of FaDOH was not significantly reduced (p>0.05) whereas the levels of FaOH and FaDOAc were significantly reduced (p<0.05) for all of the products (Fig. 8.2). Some authors have reported that the “epidermis” (root peel) of most Apiaceae root vegetables is rich in specific polyacetylenes (Czepa and Hoffmann, 2003; Kidmose et al., 2004; Baranska and Schulz, 2005) and therefore the decrease in polyacetylene content following peeling is most likely due to mechanical removal of the epidermal skin during abrasive peeling. Depending on the time of exposure to the carborundum drums and the actual load of the carrots and the texture of the carrots, the depth of peeling varies. Also, the carrot surface had become less smooth and more porous and uneven. In addition, carrot ends (tops and bottoms) were rounded during abrasive peeling, in contrast with the manual clean cut removal obtained using a knife.

The effect of abrasive peeling on the final shape of a carrot as observed in this study is illustrated in Figure 8.4. It has been reported that levels of some polyacetylenes (for example, FaOH and FaDOAc) are higher at the top and bottom ends of carrot (Czepa and Hoffmann, 2004) and as a result their levels are expected to be more affected by abrasive peeling than non-abrasive preparation. The latter might explain the fact that FaDOAc was influenced the most during peeling; the other polyacetylenes were retained in the following decreasing order: FaDOAc> FaOH> FaDOH.
As mentioned earlier, “cutting” was performed for baton and disk production prior to “washing” whereas the opposite order was followed for cubes and shred production (Fig. 8.1). This was in line with the standard industrial protocol used. As illustrated in Fig 8.2a, 8.2c, “cutting” followed by “washing” resulted in lower polyacetylene retention although the difference was significant only for disks (p<0.05) and not significant for batons (p>0.05). On the other hand, “washing” following “cutting/shredding” did not have any significant effect on the polyacetylene level (Fig. 8.2b, 8.2d). In terms of the individual polyacetylenes, FaOH, which is generally acknowledged as the most bioactive polyacetylene (Christen and Brandt, 2006) remained relatively stable during these operations.

Total polyacetylenes content (FaOH + FaDOH + FaDOAc) in the final products washed after cutting (i.e. the carrot disks and batons, Fig. 8.2a, 8.2c) exhibited the highest concentration loss, i.e. 30.2% and 44% respectively during these unit processes. It is known that cutting results in tissue damage, exposure of the cut surface area per unit volume to enzymatic activity (Huxsoll and Bolin, 1989) and may also cause mechanical release of the phytochemicals due to disruption of cells (Varoquaux and Wiley, 1994). As outlined earlier abrasive peeling of carrots resulted in a porous and uneven surface, which is indicative of cell disruption. Therefore an additional reason for the loss of polyacetylenes after peeling could have been the release of intracellular enzymes and exposure to their substrate. To minimise the impact of cutting on polyacetylene content it is recommended that the cutting blades of the industrial equipment should be as sharp as possible (Laurila and Ahvenainen, 2002). Washing of vegetables may cause leaching of beneficial components into the washing water (Watada et al., 1996; Cazor et al., 2006). However, for hydrophobic compounds such as the polyacetylenes only a small amount of loss is expected, which is confirmed by the findings of this study.

8.3.3. The effect of unit operations in minimally processed parsnips

Figure 8.3 presents levels of total and individual polyacetylenes in parsnips, from sequential sampling at different sampling points (SPs) along the processing line, as illustrated in Figure 8.1. The effect of unit operations on the level of polyacetylenes in parsnips was generally similar to those observed for carrots.
Where,


Figure 8.2. The effect of unit operations during minimal processing on the polyacetylene content of minimally processed carrots. Values refer to the polyacetylene content (µg/g dry carrot weight) of extracts determined using HPLC.

a) Baton, b) Cube c) Disk, d) Shred
Where,


**Figure 8.3.** The effect of unit operations during minimal processing on the polyacetylene content of minimally processed parsnips. a) Baton b) Disc c) Cube

Values refer to the polyacetylene content (µg/g dry parsnip weight) of extracts determined using HPLC.
Figure 8.4. Schematic Illustrating parts of carrots removed during mechanical abrasive peeling
Following peeling, a significant (p<0.05) decrease in total polyacetylene content was observed. The degree of loss over the entire process was higher in parsnips (~40%) than carrots (~29%). In parsnips, levels of FaOH were reduced by a greater amount following peeling than observed for FaDOH, which is the opposite trend to that observed for carrots. This phenomenon could have arisen from the peeling method used, as parsnips were hand peeled as a result of their softer texture rather than peeled abrasively as was the case for carrots. While it is difficult to quantify from the levels of individual polyacetylenes in Figure 8.2 compared to Figure 8.3 it would be expected that hand peeling would result in a more uniform removal of epidermal tissue than the abrasive method; the latter as discussed previously, results in the removal of large amounts of peel and other tissue from the top and bottom of the root (Figure 8.4).

In comparison with carrots, cutting and washing had a greater effect on polyacetylene content in parsnips. Similar to carrots, generation of batons and disks was carried out prior to “washing”, whereas the opposite order of processing was followed for the production of cubes. Parsnip shreds were not prepared in this study because the parsnip’s texture was incompatible with the industrial equipment and there is currently little scope for this product in the market. As illustrated in Figure 8.3 (a, b), there was a significant decrease (p<0.0.5) in the level of total polyacetylenes in the final product compared to the levels after peeling. This clearly shows that these two mechanical operations have a significant influence (p<0.0.5) on polyacetylene retention in parsnip batons and disks. A similar trend was observed for the levels of both polyacetylenes in parsnip cubes (even if the order of the operations was not the same). In terms of the individual polyacetylenes, FaOH was better retained when parsnips were cut in disks than when cut in baton and cubes. As mentioned earlier, cutting exposes the cut surface, disrupts cells, and results in nutrients and micronutrient loss including polyacetylenes. The experimental trial in the industrial setting and the type of equipment used prevented the collection of samples of the washing liquid that would allow investigation of the presence of polyacetylenes in the washing medium. Therefore the assumptions have been made regarding losses of polyacetylenes due to tissue cutting, and although these are
supported by the literature (Brandt and Rached, 2009; Rached, 2009), they should be treated with caution until proven.

8.3.4. The effect of storage in carrot and parsnips

Raw freshly cut carrot and parsnips usually have 3-4 days shelf life. Various quality and microbiological changes occur during storage (Howard and Dewi, 1996; Amanatidou et al., 2000) although little is known about polyacetylene retention (Hansen et al., 2003). In this research, the effects of storage on the retention of polyacetylenes were investigated over a 7 day storage period to monitor levels throughout and beyond the maximum expected shelf-life.

8.3.4.1. Carrots

The influence of chill storage on the levels of polyacetylenes in minimally processed carrots is shown in Figure 8.5. In carrot batons, the level of total polyacetylene was constant during the storage period, whereas in case of carrot disks, a significant decrease was observed in FaOH (65%) and FaDOAc (50%) levels. In carrot cubes, there was a significant decrease (~31%) occurred in the level of FaOH from day 0 to day 7, whereas FaDOH and FaDOAc levels were fully retained (Figure 8.5b). The highest polyacetylene retention was observed for carrot batons and the lowest retention was recorded for carrot disks, whereas an intermediate retention level to that for batons and disks was observed for shredded carrots. This may be partially explained by the lower surface area that is exposed after cutting in batons, which in turn results in less tissue breakage and subsequent leakage of nutrients, including polyacetylenes, during washing, as mentioned earlier (8.3.2). It should be noted that the shredded carrots were washed before cutting which would be expected to have a lower impact on polyacetylene losses. Overall, the observed order of retention of polyacetylenes in different carrot preparations was: batons>> cubes> shreds> disks.

It is accepted that fresh-cut processing increases the respiration rates of vegetables as a response to stress, which subsequently decreases during the chill storage period (Laurila and Ahvenainen, 2002). These physiological changes during storage may be accompanied by a reduction of metabolic activities and thus may result in higher levels of polyacetylenes (Varoquaux and Wiley, 1994; Watada et al., 1996).
Figure 8.5. Levels of polyacetylenes in carrots prepared as a) disks, b) cubes, c) disks and d) shreds over a 7 day chill storage period (4°C). Values refer to the polyacetylene content (µg/g dry carrot weight) of the extracts determined using HPLC.
Figure 8.6. Levels of polyacetylenes in parsnips prepared as a) batons, b) disks, c) cubes over a 7 day chill storage period (4°C). Values refer to the polyacetylene content (µg/g dry parsnip weight) of the extracts measured determined using HPLC.
However, polyacetylenes are phylotaxeins and, as such, would be produced by the plant in response to a stress such as cutting. In the present case, however, it would appear that the influence of lower metabolic rate outweighs that of stress-induced production of polyacetylenes (if this occurs at all).

### 8.3.4.2. Parsnips

The influence of storage on the levels of polyacetylenes in minimally processed parsnips is shown in Figure 8.6. Following storage of parsnip batons and disks the levels of polyacetylene remained unchanged however, in case of parsnip cubes, FaOH levels were significantly higher than initial values (45%, p<0.05) by day 7. In fact of the three unit operations, parsnips prepared in cubes exhibited the highest levels of FaOH following storage. The order of polyacetylene levels in the final products after 7 days storage was: cubes>batons>disks, although there were very small differences between the individual processing products. As discussed in the previous section, chill storage results in a decrease in metabolic activities and results in lower levels of polyacetylenes (Varoquaux and Wiley, 1994; Watada et al., 1996). However, as stated previously, polyacetylenes are phylotaxeins and as such are produced by the plant in response to a stress such as cutting. In contrast to the case for carrots it would appear that the influence of lower metabolic rate either equals or is less than stress-induced production of polyacetylenes.

Purified polyacetylenes are chemically unstable (Leonti et al., 2010), However given the relatively high level of retention described in some examples above, it would appear that they are generally more stable in the plant matrix. Similar results for the stability of other bioactive phytochemical such as polyphenols and antioxidants have been reported by Pacheco-Palencia et al. (2007) and Puupponen-Pimia et al. (2003). Plant material is a natural matrix with high enzyme activity and the stability of bioactive compounds varies significantly, some are relatively stable while others are highly reactive (Pacheco-Palencia et al., 2007; Gonzalez and Gonzalez, 2010).

### 8.4. Conclusions

Levels of polyacetylenes in minimally processed carrots are affected by mechanical operations (mainly during peeling as seen in section 8.3.2 and 8.3.3). Even if these
operations are essential, there is scope for optimisation of conditions during peeling (the depth of peeling relative to the exposure to carborundum drums, use of alternative peeling methods such as rubbing/brushing operations, etc), cutting (sharpness of the blades or size of cut) and washing (the time in contact with chlorinated water during washing) to potentially minimise the losses of polyacetylenes. Also when possible, washing treatment should be applied before cutting to prevent leaching of polyacetylenes. Some unit operations such as peeling and shredding may need further development to make them less rigorous (Laurila and Ahvenainen, 2002), especially abrasive peeling, as it was demonstrated in this study. In general, subsequent aerobic chill storage of carrots results in additional losses of polyacetylenes; however this is not the case for parsnips. In cases where polyacetylenes are lost during storage advances in packaging, including modified atmosphere packaging represent the next logical step in retaining bioactives such as polyacetylenes in minimally processed vegetables (Ahvenainen, 1996). All these optimisations - that could come in the form of recommendations for processors – should, however, take into account the microbiological safety, the organoleptic quality including texture as well as the equal retention of other micronutrients present in carrots and parsnips, for example vitamins, flavonoids and minerals.

8.5. References


9. Impact of frozen storage on polyacetylene content, colour and texture in carrots disks

9.1. Introduction
Refrigeration and freezing of vegetables and their derived products are of huge economic importance for both unprocessed and processed foods (Campañone et al., 2002). In fact freezing is one of the most accepted and widely used food preservation techniques for long term preservation of food is known to help in retention of key quality parameters such as sensory attributes and nutritive properties of food products over long storage periods (Fennema, 1977; Oliveira and Oliveira, 1999). Freezing retards chemical and biochemical reactions, microbial growth, water evaporation or any other process that may reduce quality or product shelf-life (Campañone et al., 2002; George, 1993). However, freezing can increase the loss of some bioactive compounds, as demonstrated for some better known bioactive compounds such as ascorbic acid, and carotenoids. For example, Favell (1998) reported a decrease in ascorbic acid due to the frozen storage of carrots for 12 months. Several studies reported a decrease in ascorbic acid (Howard et al., 1999), and carotenoids (Park, 1987; Howard et al., 1999) due to freezing of carrots. Polyacetylenes are phytoalexines, i.e. compounds synthesized by the plants in response to stress, such as fungal infections, water stress etc. Lund and White (1990) demonstrated that water stress had profound effects on the total polyacetylene content of carrots resulting in reduced levels of the three major polyacetylenes (FaOH, FaDOH, FaDOAc) and formation of other minor polyacetylenic compounds. Pre-treatments such as minimal processing, freezing may act as stress upon the plant matrices hence, can affect the metabolic activity in the plant cell and induce changes in the content and profile of polyacetylenes in the frozen samples (Chapter 8).

Freezing of fresh vegetables can be carried out by a number of methods. In a domestic situation foods are usually slow frozen whereas in industry blast freezing is usually employed. To date the effect of blast freezing on polyacetylene content has not been reported though some studies on slow freezing effect have been conducted by Hansen et al. (2003) and Kidmose et al. (2004) who reported that there was lower decrease in the levels of polyacetylenes following frozen storage of whole or cut carrots.
In addition to the bioactive content, the sensory quality of frozen food can also be influenced by the freezing method employed. For example, slow freezing may cause extensive structural damage due to the formation of larger ice crystals (George, 1993) whereas, rapid freezing using cryogenics may induce cracks as a result of an initial decrease in volume on cooling and a subsequent increase in volume due to freezing (Kalichevsky et al., 1995). Furthermore, thawing of slow frozen food products retards re-entry of extracellular ice into the cells and may cause extensive drip and texture softening (Cheftel et al., 2000). Whilst the retention of bio-active compounds during frozen storage is of paramount importance there is little point in retaining these compounds if the sensory and taste quality of the foodstuff are not also maintained. Therefore, the aim of the present study was to determine the effect of slow freezing and blast freezing, with and without prior blanching in combination with frozen storage on the polyacetylene content and quality (colour and texture) of carrot disks.

9.2. Materials and Methods

9.2.1. Chemicals
Acetonitrile (ACN), ethyl acetate and water were of HPLC grade (99.9%) and obtained from Fischer’s (Dublin, Ireland). Diatomaceous earth was obtained from Dionex (Dionex Camberley, Surrey, UK), as mentioned previously in Chapter 3.

9.2.2. Sample preparation
Fresh carrots (Daucus carota, cv. Nazri) were obtained from a local wholesaler (Donnelly’s, Dublin) and stored at 4°C for a minimum of 24 h prior to processing. Carrots with no visible damage and of root size 15.5 ± 1.5cm were selected for the processing. After hand peeling, carrots were sliced into disks (5mm) using a Berkel 800 meat slicer (Berkel company, Indiana, USA; Chapter 2, Section 2.2.2).

9.2.3. Blanching and freezing
Prior to freezing carrot disks were divided into two portions and blanching of half of each portion was carried out by immersing the carrot disks in a hot water for 75
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sec at 90°C (Hansen et al. 2003). Three batches (250 g each) of both blanched and unblanched carrots were subjected to the following two treatments:

1) Slow freezing – blanched (SFB) and unblanched (SFUB) samples were kept at -20°C and allowed to freeze gradually (12h).

2) Blast freezing – blanched (BFB) and unblanched (BFUB) samples were frozen in a blast freezer (Avon Refrigeration Co., Bristol, U.K.) operating at -30°C at an air velocity of 8m/s, until frozen (2 h) and then stored at -20°C freezer.

The samples were stored for 60 days and sampled every 15 days following which they were freeze dried (Model A6/14, Frozen in Time Ltd., England) and vacuum packed using a Vac Star 220 vacuum sealer (Vicquip Ltd., Dublin, Ireland) prior to chromatographic analysis.

9.2.4. Extraction of polyacetylenes
Extraction of polyacetylenes was performed using method described in Section 3.2.4.

9.2.5. Determination of Polyacetylene content using RP-HPLC
Separation and quantification of polyacetylenes was carried out by RP-HPLC using the method, as outlines previously in section 3.2.5.

9.2.6. Measurement of instrumental colour
The colour was measured using the method described in section 3.2.6. All the measurement was carried in triplicate.

9.2.7. Texture analysis
Texture analysis was performed using a Texture analyzer TA-XT2i, with a load cell of 25 kg. Carrot disks of the same diameter were placed under the sharp edged blade and the force (g) required to cut it versus time taken (sec) by the blade was recorded.

9.2.8. Statistical analysis
Analysis of variance was performed using SAS Statistical software package (V 9.3.1, SAS Institute, NC, USA). Data fitting was considered significant at a probability level of
95%. Means were separated by least significant difference (LSD) using the Tukey test. In total 60 samples were analyzed; 4 treatments (SFB, SFUB, BFB, BFUB) × 5 time points (day 0, 15, 30, 45, 60) × 3 replicate.

9.2.9. Degradation kinetics
The degradation kinetics of polyacetylenes during storage was investigated using the Weibull model. The Weibull model is flexible owing to the inclusion of a shape constant and has been employed to describe microbial, enzymatic and chemical degradation kinetics (Cunha et al., 1998; Manso et al., 2001; Tiwari et al., 2009).

$$\frac{C_t}{C_0} = \exp \left( -kt \right)^{\beta}$$

where \(C_t\) is the level of polyacetylenes (FaDOH, FaDOAc, and FaOH) during storage time (t, days) and, \(C_o\) is the initial level of polyacetylenes after freezing treatment (SFUB, SFB, BFUB and BFB) at t=0 days: \(k\) is the degradation rate constant (day\(^{-1}\)) and \(\beta\) (dimensionless) is the shape constant determining the shape of degradation curve. If the rate of degradation increases over time then the shape factor (\(\beta\)) is greater than 1 (i.e. formation of a shoulder). If the rate decreases over treatment time then the shape factor of the survival curve (\(\beta\)) is less than 1 (i.e. formation of a tail) and if rate is constant over time the shape factor (\(\beta\)) is 1 (i.e. first order degradation; Cullen et al., 2009).

9.3. Results and discussion

9.3.1. Effect of freezing rate on the levels of polyacetylenes
Mean levels of the 3 major polyacetylenes [falcarinol (FaOH), falcarindiol (FaDOH), and falcarindiol-3-acetate (FaDOAc)] in the fresh carrots disks were found to be 449.8, 245.2, and 100.5 µg/g dry weight (DW) basis respectively (Table 9.1). The levels reported in this work were higher than those reported in section 3.3.1, 6.3.1, 7.3.1, and 8.3.1 but within the range of those reported by Zidorn et al. (2005) for FaDOH and FaDOAC. The level of falcarinol was found to be slightly higher than that reported by Zidorn et al. (2005) and in section 3.3.1, 6.3.1, 7.3.1 and 8.3.1, but in the range of that reported by Kreutzmann et al. (2008). Levels of FaOH have previously been shown to
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vary due to several factors including cultivar type (Mercier et al., 1993), root size (Kidmose et al., 2004) and environmental and agronomic conditions (Søltoft et al., 2010).

Following blanching, the level of FaOH, and FaDOH in blast frozen carrot disks decreased from 449.8 to 402.3 µg/g DW and 245.2 to 204.7 µg/g DW, respectively, whereas FaDOAc increased from 100.5 to 128.5 µg/g DW compared to fresh control (unblanched blast frozen carrots) (Table 9.1). In case of blanched slow frozen carrot disks, the levels of FaOH and FaDOH decreased from 449.9 to 406.8 µg/g DW and 245.2 to 206.6 µg/g DW, respectively, which corroborates a previous study in Chapter 4 in which the higher temperature blanching treatments resulted in greater losses (20-25%) in the levels of polyacetylenes in parsnips. However, levels of FaDOAc were found to increase from 100.5 to 133.4 µg/g DW in blanched samples compared to unblanched ones (p<0.05). Kidmose et al. (2004) reported that blanching followed by freezing of the shredded carrots increased the levels of FaOH and decreased the levels of FaDOH and FaDOAc. Moreover thermal processing such as blanching can induce partial oxidation of FaDOH which may lead to production of FaDOAc as synthesised by Lund (1992), which may explain the increase in level of FaDOAc after blanching, as can be seen in chapter 4, that thermal processing leads to oxidation of falcarinol type polyacetylene.

Freezing method had a pronounced effect on the level of retention of polyacetylenes. For example levels of FaOH in SFUB where significantly lower than for BFUB samples (p<0.05 449.8 vs. 305.1). A similar trend was noted for FaDOAc levels (p<0.05 100.5 vs. 75.6 for SFUB vs BFUB respectively). In contrast a significant increase in the level of FaDOH from 245.2 to 416.6 µg/g DW was noted in SFUB samples compared to BFUB samples. This could be due to the conversion of FaOH to FaDOH during slow freezing process or there may be a degradation of FaOH and FaDOAc during storage. The pathway by which this can occur is outlined in Chapter 1. Kidmose et al. (2004) reported a significant decrease in FaDOH and FaDOAc during the frozen storage of carrots (Kidmose et al., 2004). No significant difference (p<0.05) were observed between the blanched slow frozen carrot disks and blanched blast frozen carrot disks for polyacetylenes this effect may be primarily attributed to blanching as discussed earlier.
Table 9.1. Quality parameters of Fresh and Frozen carrots.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Slow freezing</th>
<th>Blast freezing</th>
<th>Fresh carrot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unblanchd</td>
<td>Blanched</td>
<td>Unblanchd</td>
</tr>
<tr>
<td>FaDOH (µg/g DW)</td>
<td>416.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>206.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>245.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>FaDOAc (µg/g DW)</td>
<td>75.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>133.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>FaOH (µg/g DW)</td>
<td>305.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>406.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>449.8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>L*</td>
<td>33.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>a*</td>
<td>13.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>b*</td>
<td>16.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>TCD</td>
<td>9.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Force (N)</td>
<td>3957.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3070.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3391.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a, b, c, d</sup> Values followed by same alphabet within a row are significantly different (p<0.05).
Table 9.2. Weibull model parameters for frozen carrots during storage

<table>
<thead>
<tr>
<th>Process</th>
<th>Compound</th>
<th>$k \times 10^{-2}$</th>
<th>Shape ($\beta$)</th>
<th>RMSE</th>
<th>$R^2$</th>
<th>$R^2_{adj}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFB</td>
<td>FaDOH</td>
<td>1.06±0.32</td>
<td>0.61±0.11</td>
<td>0.058</td>
<td>0.981</td>
<td>0.961</td>
</tr>
<tr>
<td></td>
<td>FaDOAc</td>
<td>0.97±0.12</td>
<td>0.98±0.53</td>
<td>0.013</td>
<td>0.998</td>
<td>0.997</td>
</tr>
<tr>
<td></td>
<td>FaOH</td>
<td>1.16±0.48</td>
<td>1.77±1.48</td>
<td>0.016</td>
<td>0.998</td>
<td>0.995</td>
</tr>
<tr>
<td>SFUB</td>
<td>FaDOH</td>
<td>1.69±0.08</td>
<td>0.96±0.44</td>
<td>0.014</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>FaDOAc</td>
<td>0.49±0.06</td>
<td>0.66±0.18</td>
<td>0.029</td>
<td>0.986</td>
<td>0.973</td>
</tr>
<tr>
<td></td>
<td>FaOH</td>
<td>1.14±0.63</td>
<td>1.59±1.07</td>
<td>0.017</td>
<td>0.997</td>
<td>0.994</td>
</tr>
<tr>
<td>BFB</td>
<td>FaDOH</td>
<td>1.01±0.04</td>
<td>1.42±0.31</td>
<td>0.013</td>
<td>0.998</td>
<td>0.995</td>
</tr>
<tr>
<td></td>
<td>FaDOAc</td>
<td>0.59±0.07</td>
<td>1.22±0.59</td>
<td>0.005</td>
<td>0.999</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>FaOH</td>
<td>1.03±0.30</td>
<td>1.63±0.79</td>
<td>0.002</td>
<td>0.999</td>
<td>0.998</td>
</tr>
<tr>
<td>BFUB</td>
<td>FaDOH</td>
<td>1.66±0.01</td>
<td>1.89±0.35</td>
<td>0.045</td>
<td>0.994</td>
<td>0.988</td>
</tr>
<tr>
<td></td>
<td>FaDOAc</td>
<td>0.97±0.09</td>
<td>0.94±0.01</td>
<td>0.004</td>
<td>0.990</td>
<td>0.990</td>
</tr>
<tr>
<td></td>
<td>FaOH</td>
<td>0.96±0.18</td>
<td>1.09±0.13</td>
<td>0.005</td>
<td>0.990</td>
<td>0.999</td>
</tr>
</tbody>
</table>

Where:
SFB: slow frozen blanched samples
SFUB: slow frozen unblanched samples
BFB: blast frozen blanched samples
BFUB: blast frozen unblanched samples
FaDOH: falcariindiol
FaDOAc: falcariindiol-3-acetate
FaOH: falcarinol
$k$: rate constant
$\beta$: shape factor
However as mentioned earlier it should be noted that these changes in the polyacetylene content may be attributed to stress experienced by the plant, pre-treatments such as minimal processing and freezing may act as stress upon the plant matrices hence, can affect the metabolic activity in the plant cell and induce changes in the content and profile of polyacetylenes in the frozen samples (Chapter 8).

9.3.2. Effect of frozen storage on the levels of polyacetylenes

During frozen storage at -20°C the levels of FaDOH, FaDOAc, and FaOH decreased with time for all samples (Figure 9.1 and 9.2). A similar reduction in the level of all the three polyacetylenes was reported by Kidmose et al. (2004) for frozen carrots compared to refrigerated carrots stored for 4 months. The decrease in the levels of FaDOH, FaDOAc, and FaOH may be due to several changes occurring during storage and it is difficult to establish exact mechanism involved for the degradation of polyacetylenes. However, a decrease could be mainly due to enzymatic degradation and/or by some biochemical transformation among FaDOH, FaDOAc, and FaOH. Hansen et al. (2003) postulated that the reduction in the raw frozen carrots can be attributed to changes in the balance between enzymatic degradation of falcarinol and its biosynthesis. To gain a better understanding of the observed, levels of the three major polyacetylenes, levels in frozen carrot disks were examined using the Weibull model (Eq 1). The observed decrease in the level of polyacetylenes can be adequately described by Weibull model, as is evident from the high coefficient of regression ($R^2 > 0.98$) and low RMSE value ($<0.058$) (Table 9.2).

A significant difference ($p<0.05$) was observed in the degradation rate of all polyacetylenes, between blanched frozen and unblanched frozen carrot disks irrespective of the freezing method. Though blanching reduced the polyacetylene content after processing with either freezing methods (Table 9.2), however, blanched samples were more stable during frozen storage as is illustrated in Figures 9.1 and 9.2.
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Figure 9.1. Relative changes Falcarinol (●), Falcarindiol (♦) and Falcarindiol-3-Acetate (▲) during slow freezing after blanching (a) and without blanching (b).
Figure 9.2. Relative changes Falcarinol (●), Falcarindiol (♦) and Falcarindiol-3-Acetate (▲) during blast freezing after blanching (a) and without blanching (b).
Higher retention of polyacetylenes for blanched samples indicates that the polyacetylenes were more stable possibly due to removal of occluded oxygen during blanching and the inactivation of enzymes such as desaturase, acetylenase, dehydrogenase, lipoxygenase, and peroxiredutase (Minto and Blacklock, 2008), which are responsible for their degradation.

Degradation rates for FaDOH were higher in both unblanched treatements $k$ values of $1.69 \times 10^{-2}$ day$^{-1}$ and $1.66 \times 10^{-2}$ day$^{-1}$ ($R^2=0.99$) for SFUB and BFUB samples respectively. Average $k$ values for blanched samples were $\geq 10.3 \times 10^{-2}$ day$^{-1}$. In comparison to FaDOAc, FaOH had relatively high $k$ values of 1.16 and $1.14 \times 10^{-2}$ day$^{-1}$ ($R^2=0.99$ each) for SFB and SFUB samples respectively. $k$ values for FaDOAc were as low as $0.49 \times 10^{-2}$ day$^{-1}$ ($R^2 = 0.98$) for SFUB samples indicating more stability. Overall the stability of falcarinol-type polyacetylenes was in the order FaDOAc>FaOH>FaDOH (Table 9.2). The probable cause for this phenomenon may be attributable to similar reasons as suggested previously for the decrease in polyacetylene content observed during frozen storage (Hansen et al., 2003).

The shape factor ($\beta$) is a reflection of the degradation pattern of falcarinol-type polyacetylenes. The observed degradation pattern $\beta<1$ this indicates that curves followed an upward concave trajectory, indicating that there was a high degradation at the earlier storage time which decreased by the end of the storage period. A $\beta > 1$ indicates that the degradation of the molecule was lower at the start of storage period time and increased at the latter storage time period. In the slow frozen samples, shape factors for FaOH were greater than 1 indicating the degradation was highest, later in the storage period where as the opposite is true for FaDOH in slow frozen samples. In general shape factors were greater than 1 or close to it for blanched samples indicating the either degradation was greatest later in the storage period (eg., FaDOH for BFUB) or remained constant (eg., FaOH for BFB).

Falcarinol-type polyacetylenes are very unstable compounds and can be easily oxidised, dehydrogenated, and/or lose a water molecule (Chapter 4; Bohlmann et al., 1973; Leonti et al., 2010) when exposed to light, high temperature, high pressure, and particular solvents. In the present study the unblanched samples potentially may be more susceptible to changes due to presence of active enzyme system (desaturase, acetylenase,
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dehydrogenase, lipoxygenase, and peroxiredutase, Minto and Blacklock, 2008), whereas the blanched samples may be affected by heating and during the storage due to the residual activity of the enzymes in addition to the subsequent frozen storage, as suggested by Kidmose and Martens (1999). In the study described in this chapter, carrots were peeled and sliced prior to blanching and freezing. Peeling has been shown to reduce the levels of some polyacetylenes (Chapter 8) as they are mainly located in the epidermis (Czepa and Hoffmann, 2003; Kidmose et al., 2004; Baranska and Schulz, 2005), which may have had a knock on effect on retention of polyacetylenes during frozen storage in all the samples.

9.3.3. Effect of freezing and frozen storage on the Texture of carrot disks

The element of sensory quality of fresh vegetables such as carrots is their texture, which is known to correlate well with the freshness of the produce (Fuchigami et al., 1995). In the present chapter the texture of the carrot using a Warner-Bratzler test with a blade attachment was also assessed. It is generally conducted under with maximal cutting force as an attribute of the studied material quality (Drake et al., 1981; Lisinska and Gołubowska, 2006). In the present study thawed samples required a lower force than fresh samples (Figure 9.3). This finding was expected as carrots contain around 90 % water, which gives rigidity to the texture. However, when water in the cell freezes, the resulting expansion causes the cell walls to rupture, leading to softening of the texture when thawed (Schafer and Munson, 1990).

Of the two freezing type investigated, slow frozen samples required a lower force when compared to blast frozen samples. In earlier work Fuchigami et al. (1995) and later Goral and Kluza (2009) reported that rapidly frozen carrots require a higher cutting force compared to slowly frozen samples, rapid freezing induced less damage to the microstructure in comparison to the slow freezing. This is because slow freezing induces greater disruption of intracellular tissue due to the formation of larger ice crystals. Inclusion of a blanching step significantly reduced (p<0.05) the force required to cut the carrot disks, when compared to their fresh counterpart. A similar effect of blanching was observed by Tansey et al. (2010) during sous-vide processing of carrots. The decrease in
the cutting force for the blanched carrot disks may be explained by the fact that application of heat induces solubilisation of intercellular cementing pectin, which leads to cell loosening (Roeck et al., 2008).

9.3.4. Effect of freezing and frozen storage on the Colour parameters of carrot disks

The Hunter colour value parameters $L^*$, $a^*$, $b^*$, TCD were analysed for all the samples as shown in Figure 9.4. $L^*$ values for unblanched slow frozen samples (60 days) significantly increased ($p<0.05$) during storage whereas no significant changes were observed for blanched slow frozen samples as well as blast frozen samples ($p>0.05$) when compared with their respective day 0 samples. This indicates that the omission of a blanching step in slow frozen samples resulted in darkening of the samples due to enzymatic browning. The main purpose of blanching is to inactivate enzymes such as peroxidase, polyphenol oxidases and pectinmethylesterase that may cause off flavour and changes in colour. (Pala, 1983; Pizzocaro et al., 1995; Gomez and Sjoholm, 2004). Hunter’s colour parameter $a^*$ gives the idea about the redness of the sample, which in carrots is caused by the presence of the carotenoid pigment. Carrot samples at day 0 showed lower $a^*$ value compared to those of at day 60 for slow frozen unblanched samples whereas slow frozen blanched samples and blast frozen samples had statistically insignificant difference among all the samples irrespective of blanched and unblanched conditions. Similar to $a^*$ values, $b^*$ value did not show any significant difference in any of the samples irrespective of treatment and storage time. However TCD provided a more detailed picture for the changes in the colour of the thawed carrots in comparison with fresh carrot. SFUB samples yielded a significant change in the TCD values ($p<0.05$) when compared to SFB, BFUB, BFB samples, all of which did not show any significant change (Table 9.1) in this parameter. These result confirms that the colouring pigments, which in the case of carrots are primarily carotenoids, may be affected by freezing type, a similar result on the effect of storage conditions on the carotenoid content was reported by Berger et al. (2008).
Figure 9.3. Changes in the value of cutting force in frozen carrot disks with respect to storage days (Values with the same letter shows no significant difference (p<0.05))
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Figure 9.4. Changes in instrumental colour parameters of fresh and frozen (-20°C) stored carrot disks.
9.4. Conclusion
This study shows that both the inclusion of blanching and freezing method had significant impacts on the polyacetylene content of carrots. During frozen storage polyacetylenes were decreased with storage time irrespective of freezing method employed; however, the losses in samples that were blanched and stored were significantly less. Among freezing treatments, blast freezing resulted in higher retention of polyacetylenes and other quality factors such as colour and texture compared to slow freezing. Weibull degradation kinetics indicated that among the three polyacetylenes, FaDOH was the most susceptible to degradation (as demonstrated by high $k$ values) during frozen storage of unblanched carrot slices, followed by FaOH, whereas FaDOAc was the least affected. Changes in the levels of polyacetylenes during storage were supported by Weibull modelling and this approach was found to be useful in demonstrating the changes in polyacetylene content that occur during processing and / or storage of carrot disks.

9.5. References


10. General Discussion and Conclusions

Nature in itself contains solutions to many problems known to mankind, one of which is the control of certain diseases. In modern times, this observation has been backed up with various epidemiological studies demonstrating that consumption of fruits and vegetables is associated with various health benefits. Historically carrots are known as an excellent source of vitamin A and important phytochemicals such as carotenoids and flavonoids. These phytochemicals are believed to help protect against various chronic diseases such as cardiovascular disease and cancer. However, recently, researchers have identified another group of compounds known as polyacetylenes in carrots that may have health promoting properties.

Since the biological significance of C17 polyacetylenes in Apiaceae vegetables has only emerged recently (Kris-Etherton et al., 2002; Kobak-Larsen et al., 2005; Schneider and Bucar, 2005 a, b; Christensen and Brandt, 2006; Christensen and Jakobsen, 2008), information on the impact of industrial and domestic practices on their levels is lacking. Therefore, a thorough examination of the effect of both full and minimal processing strategies was warranted, particularly given the availability of emerging novel processing technologies which could be used to minimise losses of these important compounds. Consequently, the principal objective of the present work was aimed at evaluating the effects of full scale thermal, non-thermal and minimal processing on polyacetylenes content, and levels of individual polyacetylenes, in a selection of Apiaceae plant vegetables. The work attempted to address the gap in the information regarding the stability of polyacetylenes during processing. Given the strong focus of this thesis on the effect of processing on polyacetylenes in Apiaceae plant vegetables, it is appropriate to make some critical statements and recommendations based on the experimental findings for food processors. The following is a critical review of the study from this prospective.

While falcarinol-type polyacetylenes have been the subject of continuous study due to their range of potential bioactivities that could provide health benefits, it is not possible, however, to purchase commercial authenticated standards of these compounds. Therefore isolation, purification and identification of these compounds from their native source are essential steps for quantification and evaluation of their
bioactivity. Consequently, the initial part of this research consisted of a study on the isolation, purification and characterisation of the target polyacetylenes from carrots using liquid chromatography coupled to negative ion tandem mass spectrometry (Chapter 2) to provide purified standards that could be used to evaluate the effects of processing technologies quantitatively.

The focus then was to evaluate the polyacetylene content following thermal processing of Apiaceae vegetables (carrots, parsnips, and fennel) and to assess the degradation products formed during the processing methodologies employed (Chapters 3, 4, and 5). The findings are of significance to sectors of the food industry involved in processing of these vegetables, as well as to the consumer of processed products and consumers that conduct processing in a domestic setting. Novel processing technologies such as ultrasound-assisted drying and high pressure-temperature processing were also investigated (Chapters 6 and 7). The final aspect of the research focused on the investigation of polyacetylene content during minimal processing of carrot and parsnip in an industrial setting as reported in Chapter 8, followed by an evaluation of frozen storage on the levels of polyacetylenes in carrots (Chapter 9). Both of these chapters are of particular significance for industry. The following paragraphs present a summary of the major outcomes of these processing research themes.

In many cases, Apiaceae vegetables such as carrots are still processed using traditional practices, such as immersion in hot water. This technique had an unexpected effect on polyacetylene levels; for example, at higher temperatures the contents of polyacetylenes increased with increasing processing temperature. This was particularly apparent for levels of FaOH, the polyacetylene with the most extensive and convincing evidence for a health promoting effect. The reason behind increased levels of polyacetylenes at higher temperature remains unclear. However, evidence provided for other phytochemicals suggests that this effect could be a result of the increased extractability of the compounds, as heat induces solubilisation of the intercellular cementing pectin, thus facilitating cell loosening (Waldron, 2004; Roeck et al., 2008). In addition, a general increase in polyacetylene levels with longer holding times was observed. Although this behaviour was unexpected, it could be attributed to the leaching of soluble solids from the carrot matrix (which would reduce the overall solids content) in combination with retention of
polyacetylenes, as these are largely insoluble in water. As a result, the dry weight polyacetylene levels appear to increase. Further studies are needed to confirm this hypothesis, which will be discussed later. At lower temperatures of 50-60°C, a significant decrease in the levels of FaOH and FaDOH for all holding times (2-60 min) was observed, with respect to raw unprocessed samples. The behaviour of polyacetylenes with respect to heat processing, especially at lower temperatures, is further complicated by the fact that FaOH is a precursor of both the FaDOH and the FaDOAc and, therefore, in the presence of active enzyme systems, these compounds may be inter-converted (Kidmose et al., 2004). It should also be noted that FaDOH is the main compound responsible for the bitter off-taste in carrots (Czepa and Hofmann, 2004; Kreutzmann et al., 2007).

Most food processors do not have access to the sophisticated analytical equipment required to determine levels of polyacetylenes and then optimise their procedures for their retention. However the present study demonstrated that mathematical modelling offers good potential for predicting polyacetylene levels. In the present work, a mathematical model was developed that is capable of predicting polyacetylene content, and different combinations of L*, a*, b* at different holding times and treatment temperatures, using polynomial regression analysis of water immersion processed carrots. Mathematical and graphical model performance indices indicated that it had acceptable predictive performance. The coefficient of determination values ($R^2$) for predicted FaOH, FaDOH, FaDOAc, and colour parameters, showed good correlation with the experimental data at a 95% confidence level and indicated that second order polynomial regression models could be employed to predict these quality parameters. The models could aid food processors in the optimisation of critical operating parameters (holding time) for desired product quality attributes.

Though water immersion cooking is a commonly used thermal processing method, there are other thermal processing methods about which little is known. *Sous-vide* (SV) processing is a novel thermal processing technology with the potential to retain the polyacetylene content and colour of thermally processed Apiaceae vegetables, while imparting a longer shelf life than water immersion processing. Blanching is commonly implemented prior to SV processing in order to inactivate enzymes that might otherwise lead to deterioration in quality. In the present study, it has been demonstrated that blanching has the greatest negative influence on the retention of polyacetylenes in SV processed parsnip disks and
results in significant decreases in falcarinol and falcarindiol. However, subsequent SV processing did not result in additional significant losses in polyacetylene levels in the pre-blanched samples. The decrease in the levels of polyacetylenes following thermal processing can be attributed to their thermolabile nature.

Subsequent anaerobic storage of SV processed samples for 21 days resulted in a significantly higher retention of polyacetylenes compared to water immersion processed samples stored in aerobic conditions for 5 days. Liquid chromatography and accurate mass mass-spectrometry analysis revealed that degradation of falcarinol-type polyacetylenes, following thermal processing, may be a result of oxidation and dehydrogenation, which yield the oxidized forms of falcarinol-type polyacetylenes, i.e. falcarindione, dehydrofalcarinol and dehydrofalcarinone. This research demonstrated that compounds that could have arisen from both the oxidation and dehydrogenation of polyacetylenes were present in processed parsnip samples, but were absent or very low in amount compared to their fresh counterparts. Consequently, the research has revealed the importance of the type of storage used, post-processing, in order to stabilise and maximise retention of the target polyacetylenes (FaOH, FaDOH and FaDOAc).

In line with observations from the SV study, domestic practices (boiling and roasting) resulted in losses in all the three polyacetylenes from fennel bulbs, when the calculations were performed on a fresh weight basis and took into account the leaching effect of soluble solids. Similar to polyacetylenes, polyphenols and antioxidant activity decreased following thermal processing. A decrease in the levels of polyphenols during thermal treatment may be due to heat-induced chemical oxidation and leaching of water-soluble polyphenols during boiling, as reported by several authors (Roy et al., 2007; Podsedek et al., 2008). In particular, during roasting, Maillard reactions may contribute to a reduction of polyphenols and antioxidant activity. Following roasting, a substantial increase in the levels of ferulic acid was observed, which may be attributed to the degradation of caffeic acid, a precursor of ferulic acid (Boerjan et al., 2003; Rechner et al., 2001). A probable hypothesis is that, through the Maillard reaction, caffeic acid is O-methylated to ferulic acid. The fact that levels of total phenols and the majority of individual polyphenols decreased correlates with antioxidant activity values and suggests that phenolics may be the major contributor to the total antioxidant activity of the samples tested. Roasted samples of fennel showed the presence of a potentially
harmful compound HMF. This phenomenon can be explained by the fact that fennel bulb is rich in sugars and during roasting formation of HMF may take place, via the Maillard reaction between reducing sugars and amino acids.

The most notable trend in the marketing of fresh vegetable in the past decade is the emergence of minimally-processed, fresh-cut products, which are sold from chilled retail display units. For example carrots and parsnips are often consumed as minimally processed ready-to-eat convenient foods. Therefore an evaluation of the influence of unit operations such as peeling and cutting on the levels of polyacetylenes in minimally processed carrots and parsnips was carried out. The results showed that initial unit operations (mainly peeling) have the most influence on polyacetylene retention. This finding can be attributed to the high polyacetylene content of the vegetable peels. In most cases, when washing was performed after cutting, lower retention was observed, possibly due to leakage during tissue damage incurred in the cutting step. The relatively high retention during storage indicates high plant matrix stability. Comparing the behaviour of polyacetylenes in the two vegetables during storage, results showed slightly better retention in parsnips than in carrots. Overall, the results suggest that unit operations, especially abrasive peeling, may need further optimisation to minimise bioactive polyacetylene losses.

A significant proportion of vegetables such as carrots and parsnips are sold as frozen goods. Hence the effect of freezing method (slow or blast freezing) with or without blanching and storage at -20°C on the levels of three polyacetylenes (FaOH, FaDOH, FaDOAc) in carrot disks was evaluated. The eating quality of the carrot disks was also assessed using instrumental texture and colour measurements. Blast frozen carrot disks retained higher levels of polyacetylenes compared to their slow frozen counterparts. Whilst the levels of retention of total polyacetylenes was higher in unblanched disks relative to blanched disks prior to freezing, a sharp decrease in the levels of polyacetylenes was noted in unblanched, frozen carrots during a 60 day storage period at -20°C. FaDOH was the most susceptible to degradation during frozen storage of unblanched carrot slices, followed by FaOH and FaDOAc. The changes in the levels of polyacetylenes during storage were adequately described by the Weibull model function. The texture and colour were also found to decrease during frozen storage in comparison to fresh carrots.
Apiaceae vegetables are often sold as ingredients in other products, in particular in dehydrated soups. They could also be sold in dehydrated form as healthy snack. To offset enzyme-induced quality losses, carrots and parsnips are blanched prior to drying. This blanching step is usually performed using simple water immersion. However ultrasound has emerged as a promising technique for retaining potentially bio-active components. Hence the effects of ultrasound and blanching pretreatments on polyacetylene (falcainol, falcacid and falcainiol-3-acetate) and carotenoid compounds of hot air and freeze dried carrot disks were investigated. Ultrasound pretreatment followed by hot air drying (UPHD) and freeze drying resulted in higher retention of polyacetylenes and carotenoids in dried carrot disks than in respective replica samples dried in conventional manner. The observed increase in the retention of polyacetylenes may arise from an increase in the extractability of the compounds, while the different effects observed for different holding times could be a reflection of the activation energy required to dissociate polyacetylenes from pectin-rich cell walls. As suggested by various authors, improved extraction efficiency following sonication can be attributed to the propagation of ultrasound pressure waves, induced cavitation and high shear forces resulting in increased mass transfer (Jian-Bing et al., 2006; Vilkhu et al., 2008). Some authors have suggested that the degassing effect observed under sonication may be similar to that observed under vacuum treatment, which can enhance diffusion into pores on the surface and may explain the enhanced extractability (Simal et al., 1998). The colour parameters strongly correlated with carotenoids (p<0.05). The research in his thesis has shown that ultrasound pretreatment is a potential alternative to conventional blanching treatment in the preparation of dessicated forms of carrots.

As a result of increasing knowledge with regard to the possible negative impact of traditional thermal processing techniques on thermally-labile, health-promoting compounds, a range of non-thermal alternative processing strategies have emerged. Of these, high hydrostatic pressure processing appears to be the most promising because of its ability to inactivate micro-organisms, yet have limited or no effect on covalent bonds in small bioactive molecules. Indeed this technology is now the most widely employed in industry. The ability of high pressure treatment to preserve foods has been known for some time. The present study assessed the effect
of high pressure-temperature processing on the levels of three polyacetylenes (FaOH, FaDOH, FaDOAc) in carrot disks immediately after processing in comparison to SV processing. The degradation kinetics of these compounds, following processing at high pressure-temperature, was also investigated. The highest pressure-temperature combination which gave maximum retention at the 10-30 min treatment time investigated was for FaOH, 400 MPa at 60 and 50°C for 10 min; for FaDOH, 400 MPa, at 50°C for 10 min and for FaDOAc, 400 MPa, at 50°C for 10 min, respectively. FaDOAc was found to be most barosensitive and FaDOH was found to be most thermosensitive of the three polyacetylenes studied. When compared with SV processed carrot disks, high pressure temperature processed samples resulted in better retention of polyacetylenes. This phenomenon may be attributed to the limited effect of high pressure (at moderate temperatures) on covalent bonds, as suggested by some authors (Balny et al., 1997). Moreover, high pressure treatment also increases the extractability of the phytochemicals from the plant matrix making these compounds more bioavailable, which may also contribute to the relatively high retention of polyacetylenes in high pressure processed samples compared to SV processed samples (De Ancos et al., 2002; Oey et al., 2008). A mathematical model capable of explaining the changes in the degradation rate of polyacetylenes at different pressures, temperatures and times was developed using the Weibull model function. Mathematical and graphical model performance indices indicated that this model had acceptable performance. The model had a high $R^2$ value and low $RMSE$ values indicating a good correlation with the experimental data at 95% confidence level. The findings suggest that Weibull model distribution could be employed to describe the changes in the levels of polyacetylenes during high pressure-temperature processing. The model could aid food processors in the optimisation of critical operating parameters (treatment time) to achieve desired product quality attributes.

In order to confirm the presence and structural identity of polyacetylenes in Apiaceae vegetable, the potential of negative ESI-MS and MS/MS for structural determination of polyacetylenes aided by the accurate mass measurements, was investigated in Chapter 2. The major advantages of this method are: low background interference and the fact that it by-passes MS/MS on sodiated molecular ions which are inherently difficult to fragment under low collision energy. The technique also avoids the derivatisation steps, which may be required in conventional EI ionisation.
The CID spectra showed that the falcarinol-type polyacetylenes generally underwent fragmentation in two different pathways: one that was initiated with the cleavage of C7-C8 bond (m/z 105.0, m/z 105.0/107.1 and m/z 147.0/149.1) found in falcarinol, falcarindiol and falcarindiol-3-acetate respectively, and the other with the cleavage of C3-C4 bond producing m/z 187.1 from falcarinol, and m/z 203.1 from falcarindiol and falcarindiol-3-acetate. The ‘MS3’ experiments on m/z 203.1 from falcarindiol (and falcarindiol-3-acetate) confirmed cleavage of the C7-C8 bond. Furthermore, the ‘MS3’ data established that the loss of alkyl (pentyl and hexyl) radicals occurred during CID of deprotonated falcarinol-type polyacetylenes; this result also revealed that loss of largest alkyl group (hexyl) in polyacetylenes occurred by homolytic cleavage of C11-C12 bond. The observation of hydrogenation reaction(s) with falcarindiol and falcarindiol-3-acetate, i.e. ion peaks at m/z 105.0/107.1 and m/z 147.0/149.1, suggested that the reaction(s) is/are charge-assisted, while the m/z 105.0 in the CID mass spectrum of falcarinol implied that a charge-remote mechanism operated in the fragmentation of falcarinol. Following CID, all of the polyacetylenes produced abundant ion peaks that were useful for structural confirmation, particularly via the cleavage of C3-C4, C7-C8 and C11-C12 bonds. The research has shown that this approach has the potential to be implemented in the MRM-methods for quantitative analysis of falcarinol type polyacetylenes. In addition, the fragmentation patterns observed could inform similar studies on unrelated polyacetylenes from other species, such as marine sponges. Furthermore the optimised LC-MS condition showed baseline separation of the common polyacetylenes in crude extracts of carrots within 35 minutes. This is an improvement in comparison to the LC methods described in literature where separation times between 45 and 95 min were required. Thus, this work also provided an important analytical development. Relatively few reports on the use of ESI-MS for the analysis of polyacetylenes exist and all but one of these uses ESI-MS in the positive ion mode. The previous report involving use of ESI-MS in the negative ion mode could not detect purified falcarinol Pferschy-Wenzig et al. (2009). However, the systematic approach used in this work in developing ESI-MS in the negative ion for the analysis of falcarinol-type polyacetylphenes has clearly demonstrated and validated its potential as a sensitive and reliable tool, for scientific and industrial communities, for the analysis of these compounds.
In summary, the research reported in this thesis has shown that polyacetylenes in Apiaceae vegetable are significantly affected by different types of processing. Great potential exists to retain the levels of these important compounds in processed vegetables by using novel processing technologies, such as ultrasound and high pressure. However, the need for further research to optimise these processing technologies exists and these could come in the form of recommendations for the food industry.

Future work
1) To study the effects of processing on bioavailability and potency in the diet as well as circulation levels/circulatory forms and half life.
2) Effects on gut microflora (invitro and invivo studies) and stability in the GIT (i.e. effects of pH and GIT conditions, enzymes).
3) To investigate possible microbial transformation of the polyacetylene compounds using ESI-MS.
4) To investigate the changes incurred in polyacetylenes during novel processing (such as ultrasound, high pressure, pulsed electric field, UV) techniques on a molecular level and study the products formed, using ESI-MS.

References


11. Recommendations to the Industry

A major focus of the present work was to assess the impact of commonly employed processing steps on polyacetylene levels in vegetables with a view to providing recommendations to processors for maximum retention of polyacetylenes. The project involved examining the effects of industrially relevant protocols for a selection of vegetables (carrots, parsnips and fennel), incorporating pre-processing steps such as blanching. Both thermal (water immersion, sous-vide and roasting), minimal and non-thermal methods (high pressure processing, ultrasound) were examined as well as combinations of same (for example, thermal high pressure treatments and ultrasound assisted drying). The points raised below are intended to provide clear guidance to processors as to the impact of processing on polyacetylene levels and outline some steps that can be taken to maximise retention of these important compounds.

- The inclusion of a blanching step prior to sous-vide (SV) processing results in a significant decrease in levels of FaOH and FaDOH in parsnip disks. Subsequent SV processing had little effect on levels of polyacetylenes, however, chill storage for up 20 days did result in significant decreases in these compounds. The omission of the blanching step may not be practical in some cases, especially if there is a significant lag between the preparation of the uncooked disks and SV processing. However, if processors and retailers desire to maximize polyacetylenes in SV processed parsnip disks, storage for periods in excess of 20 days is not recommended.

- Roasting results in significant losses of polyacetylenes from fennel bulbs. Losses are less dramatic when the bulbs are boiled; nonetheless, levels in boiled bulbs are much lower than in fresh bulbs. Therefore if processors desire to produce thermally processed fennel bulbs, boiling is the recommended technique for maximizing polyacetylene content.

- During minimal processing, abrasive peeling accounts for most of the losses in polyacetylene levels, when compared to other minimal processing treatments such as cutting and washing. Therefore, to maximise polyacetylene contents in
minimally processed carrot products less severe methods of peeling are recommended.

- Washing performed after cutting retains lower levels of polyacetylenes, possibly due to tissue damage by cutting during minimal processing.
- Polyacetylenes are stable in a respiring plant matrix when stored for a short term (7 days) in chill storage following minimal processing. The use of MAP packaging preserves polyacetylene content, but does not give significantly better retention than storage in air.
- Blast freezing and storage at -18°C is a good technique for preserving the polyacetylene content of carrots, provided the samples are blanched prior to freezing.
- Ultrasound assisted hot air drying (UAHD) resulted in higher retention of polyacetylenes in dried carrot disks than blanching followed by hot air drying. Given the minimal impact of ultrasound on polyacetylene content and the general negative impact of blanching, ultrasound could be considered as a replacement for blanching.
- High hydrostatic pressure processing (400-500 MPa) in combination with temperatures (50-70°C) for short duration (5-10 min) provides an excellent food processing technology, which has the potential to retain polyacetylenes in carrots in contrast to conventional thermal processing.
- Polynomial regression models have good potential for predicting the effect of time/temperature regimes on the polyacetylene content and instrumental colour of carrots. These models could aid food processors in optimising critical operating parameters (holding time) for desired product quality attributes.
**Publications**

**Peer Reviewed Publication**


**Book Chapter**

Publications (Conference proceedings/ Abstracts)

International


Institute of Food Technologists Annual Meeting, Chicago, USA, from July 17-20, 2010.


National


3) Rawson, A., Brunton N., and Tuohy M. (2008). Effect of extraction method, processing and storage on the levels of key polyacetylenes from carrot root
(Daucus Carota) In: Annual Student Seminar, Ashtown Food Research Centre, Ashtown, Dublin, p 16.


6) **Rawson, A., Chacko, J., Yadav, Y.K.,** (2003). Dehydration of Mushrooms by a Tray Drier’, poster presented at the 37th ISAE convention held at Maharana Pratap University of Agriculture and Technology, Udaipur, India.

**Industry Blueprint**


**Workshops**

Appendices

A1. Falcarinol 1 H, NMR Spectra
A2. Falcarinol 13 C, NMR Spectra
A3. Falcarindiol 1 H, NMR Spectra
A4. Falcarindiol 13 C, NMR Spectra
A5. Falcarindiol-3-acetate impure 1 H, NMR Spectra
A6. UV absorbance characteristics of falcarinol, falcarindiol, and falcarindiol-3-acetate

Measurements on the Shimadzu were performed with the below parameters

Mode: Photometry- Spectrum
Measure: ABS
Scanning: 600-200
Rec: 0-2A
Speed: medium
Scan no.: 1
Display: sequential

1) Falcarinol (against acetonitrile in different dilutions)

![Characteristic ultraviolet spectra of Falcarinol](image)
<table>
<thead>
<tr>
<th>Compound</th>
<th>Dilution factor</th>
<th>Concentration Mol/L</th>
<th>λ (nm)</th>
<th>Absorbance</th>
<th>ε (Lmol⁻¹cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FaOH</td>
<td>1:2</td>
<td>0.003643</td>
<td>256.5</td>
<td>1.114</td>
<td>306</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>243.0</td>
<td>2.060</td>
<td>566</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>231.0</td>
<td>2.280</td>
<td>626</td>
</tr>
<tr>
<td>FaDOH</td>
<td>1:1</td>
<td>0.000872</td>
<td>284.0</td>
<td>0.264</td>
<td>303</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>258.0</td>
<td>0.669</td>
<td>767</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>245.0</td>
<td>1.043</td>
<td>1196</td>
</tr>
<tr>
<td>FaDOAc</td>
<td>1:1</td>
<td>0.00221</td>
<td>284.5</td>
<td>0.183</td>
<td>83</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>260.0</td>
<td>0.532</td>
<td>241</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>246.5</td>
<td>0.812</td>
<td>367</td>
</tr>
</tbody>
</table>

Comparison with the literature: For FaOH the above values are only a bit below the reported values by Hansen and Boll, 1986, Phytochemistry Vol. 25 (2), 529-530.
A7. Negative ion CID mass spectrum of the dehydrated falcarinol ions, [M–H₂O–H]⁻. The fragment ion m/z 141.1 supports that the hexyl moiety is lost from the deprotonated falcarinol.
A8. Comparison between extraction methods: Accelerated solvent extraction (ASE) vs. solid-liquid extraction
And optimisation of ASE extraction method.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Extraction method</th>
<th>[polyacetylene], mg/g DW</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Falcarinol</td>
<td>ASE</td>
<td>697.99</td>
<td>8.00</td>
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<tr>
<td>Falcarindiol</td>
<td>ASE</td>
<td>638.09</td>
<td>6.76</td>
</tr>
<tr>
<td>Falcarindiol-3-acetate</td>
<td>ASE</td>
<td>380.75</td>
<td>7.74</td>
</tr>
<tr>
<td>Falcarinol</td>
<td>Sold/Liquid</td>
<td>517.05</td>
<td>10.74</td>
</tr>
<tr>
<td>Falcarindiol</td>
<td>Sold/Liquid</td>
<td>456.49</td>
<td>16.10</td>
</tr>
<tr>
<td>Falcarindiol-3-acetate</td>
<td>Sold/Liquid</td>
<td>430.35</td>
<td>44.84</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Extraction temp, °C</th>
<th>Falcarinol</th>
<th>Falcarindiol</th>
<th>Falcarindiol-3-acetate</th>
<th>Total polyacetylenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>574.696061</td>
<td>610.9573835</td>
<td>318.7297762</td>
<td>1504.383221</td>
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<tr>
<td>60</td>
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<td>590.6204163</td>
<td>370.4135145</td>
<td>1665.525281</td>
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<td>1701.176208</td>
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<tr>
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<td>667.2793469</td>
<td>545.2685828</td>
<td>345.8213385</td>
<td>1558.369268</td>
</tr>
</tbody>
</table>
A9. Validation of the model

a) Residual is randomly distributed
b) Normality assumption satisfied as Residual plot is a straight line

Residual Versus Run Order

Normal Probability Plot
FaDOH

Residual Versus Run Order
(FaDOH)

Observation Order

Residual

Normal Probability Plot
(FaDOH)

Percent

Residual
FaDOAc

Residual Versus Run Order
(FaDOAc)

Normal Probability Plot
(FaDOAc)
A10. Antioxidant activity of Polyacetylenes

Antioxidant activity of the three polyacetylene molecules isolated from carrot extracts using the FRAP and DPPH assay.

Antioxidant activity of the three polyacetylene molecules isolated from carrot extracts using the ORAC assay.